CONTENTS

I. REVIEW OF PROTOZOOLOGY AND SPIROCHAETOSIS

MALARIA

EPIDEMIOLOGY

The incidence of malaria depends on several important factors such as, rainfall, season, altitude, breeding place, subsoil water, malarial parasite, gametocyte carriers and anopheline vectors.

Rainfall, when excessive, is indirectly responsible for incidence of malaria as it provides ideal breeding places for anopheline mosquitoes. This was demonstrated by Covell and Singh (1943), Adhikary (1944), Viswanathan (1946). Season, on the other hand, exerts its influence in a different way. It alters malarial incidence by directly affecting the breeding of mosquitoes, the latter being very sensitive to seasonal change of atmospheric temperature and humidity (Russell and Rao, 1941, Pal, Rajindar, 1945 *a*, *b*) Of the two factors, temperature and humidity, the mosquitoes reach more to change of temperature than humidity. But when exposed to different temperatures alone, the main reaction is avoidance of high temperatures, (Thompson, 1941). With the change of season the density, biting propensity as well as the infectivity of the mosquitoes also change. Regarding density, Afridi *et al* (1940) showed that in the case of *A. culicifacies* not only the relative numbers but also the proportions between the young and aged adults vary with change of season Similar observations were also made by Russell and Rao (1941). The probable reasons for the seasonal variation about the biting propensity and the infectivity of the mosquitoes were studied by White and Narayana (1940) and Strickland *et al*. (1939).

Transmission of malaria ceases at high altitudes varying between 6,300 feet and 7,000 feet (Wynter-Polyth, 1944, Capon, 1940). The vector species are abundant up to a height of 4,000 feet, but sometimes when the temperature is unusually high these may appear in places as high as 5,500 feet (Russell and Jacob, 1942). Besides altitude, physiography of the land may have some influence on the incidence of malaria. This was suggested by Strickland (1939).

When breeding places are available in plenty and they satisfy the conditions necessary for profuse breeding, epidemics of malaria are encouraged. Russell and Rao (1942) have shown that on the availability of the breeding places depended the density of mosquitoes. Besides availability, production of progeny by the mosquitoes is influenced by some features of the breeding place The features are shade, movement of water, temperature and composition of water. Thus while the ovipositing females of *A. minimus* are attracted by shade (Thomson, 1940), those of *A. culicifacies* are obstructed when the shade is due to rice plants grown more than 12" in height (Russell and Rao, 1942). Although *A. minimus* prefers still water for breeding, yet shade is more important than movement of water. This has been proved by Thomson (1940).

High temperature kills the larvae of many anopheles mosquitoes. Thus a temperature of 41°C kills larvae of *A. minimus* within 5 min, 43.5°C kills those of *A. hyrcanus* and *A. barbirostris*, while at 45°C the larvae of *A. vagus* remain unaffected At many places in India the daytime temperature of shallow stagnant water repeatedly exceeds 41°C and in this way prevents breeding of *A. minimus* (Thomson, 1940). Lower temperatures on the other hand, are not so injurious. At temperatures as low as 16°C the development of eggs, larvae and pupae continue and adults emerge in considerable numbers (Thomson, 1940).

Regarding the composition of water, the pollution caused by organic matters such as, vegetables and silt and progressive increase of amorphous organic substances results in the production of conditions unfavourable for the breeding of mosquitoes (Thomson, 1941); Russell and Rao, 1942). There are some aquatic plants, some of which encourage while others inhibit the breeding of several species of mosquitoes. Thus *A ramsyi* while rare or absent among most plants, breeds profusely in presence of Pistia; breeding of *A annularis* is inhibited by duck weeds (Lemna) but encouraged by *Hydrilla verticellata* (Sen, 1941). How these plants affect the breeding of mosquitoes and whether they alter the composition of the water of the breeding places is not known.

Rice field, irrigation and soil erosion provide places for breeding of anopheline mosquitoes Breeding in rice fields depends on the stage of growth of rice plants, and on the conditions of rice fields such as those occurring during transplantation, harvesting etc. (Russell and Rao, 1940, Sen, 1948). When constructions are faulty, irrigation systems lead to profuse breeding of the vector species (Abraham and Samuels, 1944, Rao, 1945; Covell, 1946) Soil erosion itself and measures adopted to control it also give rise to the formation of breeding grounds (Rep. Madras Govt., 1948) Of all types of breeding places found in India the majority have been created by man himself (Henderson, 1949).

Regarding the role of subsoil water, it may be said that a high level of subsoil water is detrimental to the breeding of *A philippinensis*. This conclusion was reached by Iyengar (1942, 1944) and Jafar and Iyengar (1947).

The severity of an epidemic depends on the species of malarial parasites prevailing. All severe and extensive epidemics have been found to have been caused by *P. falciparum* parasites (Gilroy, 1939, Viswanathan, 1945; Das Gupta and Siddons, 1945; De Burca, 1946). But where malaria is mild and irregular, the offending parasite is either *P. vivax* or *P. malariae* especially the latter (Singh and Jacob, 1944, Rao *et al*, 1946; Abraham and Samuels, 1944).

For successful transmission of malaria, presence of gametocyte carriers and right type of anopheline vectors are essential. An increase in the number of *gametocyte carriers*, especially the carriers of *P. falciparum* predisposes to high rise of the

incidence of malaria (Basu, 1947*a*). In a given locality several species of anopheles mosquitoes may be found but all of them, however, do not serve as vector. Again the same species which is dangerous at one place may be quite harmless at another. The following is the distribution of anopheline mosquitoes in different parts of India :

Distribution of Anopheles Mosquito

Bengal : There are 31 species of anopheles mosquitoes in Bengal of which *A minimus*, *A sundaicus* and *A philippinensis* are most important, and *A. culicifacies* the least (Sen, 1940). The anophelines in Bengal show zonal distributions; thus (i) *A. philippinensis* in the deltaic regions of lower Bengal (Iyengar, 1944) and at the border between, Bengal and Orissa south of Kharagpur (Ganguli, 1947); (*ii*) *A minimus at the foot hill (Iyengar, 1939) in tea gardens in* Jalpaiguri and Darjeeling (Sen, 1948*b*, Gilroy, 1939), (iii) *A stephensi* both type form and var *mysorensis* in Calcutta (White, 1940*a*; *Siddons*, 1946) ; (iv) *A. sundaicus* in Calcutta and its suburbs including the salt lake area (Sen, 1948); (v) *A varuna* in Bally mill areas close to Calcutta (Roy, 1939); and (vi) *A. jeyporiensis* and *A leucosphyrus* at the border between Bengal and the Arakan district of Burma Here *A sundaicus* and *A philippinensis* do not play significant parts in transmission of malaria (Macan, 1950)

Assam. In Assam *A. minimus* serves as the most important vector (Lamprell, 1940, Assam Med. Res. Soc, 1931-41, Viswanathan *et al.*, 1941). But in the forest areas close to the oil wells of Digboi malaria is transmitted exclusively by *A leucosphyrus* which is a jungle species (Clark and Chaudhury, 1941). Mortimer (1946) has studied anopheline fauna at Monipur.

South India In Nilgiris, Wynnad and southern Travancore *A fluviatilis* is the vector. In the last named place *A. varuna* and *A. culicifacies* may sometimes be found naturally infected (Covell and Harbhagwan, 1939, Mathew, 1939, Russell and Jacob, 1942) *A culicifacies* transmits malaria in Pattukkoti Taluk, Tanjore District (Russell and Rao, 1941); in Ennore-nellore coastal area (Russell and Jacob, 1939*a*) and in villages around Tungabhadra project area (Rao *et al.*, 1946). In some places on the north coast of Madras *A. stephensi*, while in others *A. sundaicus* is the offending species (White *et al*, 1947). The latter is important in places around Vizagapatam (White and Rao, 1943). In South Eastern India *A. subpictus* and *A. vagus may be found naturally infected* (Russell *et al.*, 1939). Subramanian and Chetty (1949) carried out survey on malaria in Tirumalai village, Chotter District, Madras.

Orissa : The principal vector in the plains of Orissa, inland from the coast is *A. annularis*, but near the sea board including the places around Chilka lake *A. sundaicus* is more dangerous (White and Adhikari, 1939; Covell and Singh,

1942; White and Rao, 1946*a*; Venkat Rao, 1949). In the hilly parts, *A. fluviatilis*, *A. varuna*, *A. minimus* and in some places on the coast *A. aconitus* and *A. annularis* may sometime serve as the vectors (Venkat Rao, 1949; White *et al.*, 1943).

Central Province : Previously *A. pallidus* was thought to be the vector in Udaipur State (Roy and Biswas, 1942), but later it was shown that the real vector was *A. fluviatilis* (Subramanian and Sen Gupta, 1950). In other places in the Central Province excluding the jungle villages, *A. culicifacies* and *A. fluviatilis* while in jungle villages *A. theobaldi* transmit malaria (Subramanian and Dixit, 1948).

Mysore . The two most dangerous species in the state of Mysore are *A. culicifacies* and *A. fluviatilis*. In the towns of Mysore and Bangalore *A. stephensi*, however, is alone responsible (Rao, B. A., 1945, Rao and Nassiruddin, 1945; Rao, B. A., 1948).

Bihar : The distribution of anopheline vectors in Bihar is as follows : in Hazaribagh range, *A fluviatilis*, in Ranchi plateau, *A culicifacies*, in Jharsuguda, *A. stephensi*; and in Singhbhum hills *funestus* group (White, 1943; White and Narayana, 1940). Malaria in Jharia mining settlement was studied by Rao (1944).

Beluchistan . *A. superpictus* is the most prevailing species in Quetta cantonment (De Burca, 1939), but in the villages around Quetta *A. superpictus*, *A. culicifacies* and *A. stephensi* serve as vectors (Afridi and Bhatia, 1947). In Fort Saudeman malaria is transmitted principally by *A culicifacies* (De Burca, 1946*b* and De Burca and Jacob, 1947).

In East Central India, Bombay, Hyderabad and Salsette island which is close to Bombay *A fluviatilis* is the offending species (White, 1946*d*; Singh and Jacob, 1944; Abraham and Samuels, 1944; Nair, 1949). However, in U.P. and Delhi, *A minimus* and *A. culicifacies* respectively, while in Satpura range, *A. varuna* and *A fluviatilis* take part in spreading malaria (Rep. Mal. Inst., India, for the year 1938, Pal, Rajindar, 1943, White and Adhikari, 1940). Malaria in Bijapore was studied by Godbole *et al* (1948) Other factors which contribute to the epidemics of malaria are rise of population due to arrival of evacuees, refugees, labourers, etc , starvation, malnutrition and exhaustion (Das, 1943; Covell and Singh, 1943; Chaudhuri and Rai Chaudhuri, 1945).

Forecast of epidemics · In forecasting the epidemics of malaria in the Punjab, the method first described by Gill has been found very helpful and precise (Yacob and Swaroop, 1945). Rainfall bears a fair degree of relationship with subsequent epidemic incidences, but not the fluctuations of spleen rate (Yacob and Swaroop, 1947*a*; Swaroop, 1949). Epidemics occur in the Punjab at intervals of 8 years, while in Orissa these take place once every 4 to 6 years (Yacob and Swaroop, 1945; Venkat Rao, 1949).

Transmission season : This is usually determined by noting the extent of malaria in infants (Viswanathan, 1943). Viswanathan (1941*b*) considered that infants of ages not more than two but less than four months furnish best evidences of seasonal infections. The extent of malaria may also be estimated from the incidence of malaria in infants (Russell *et al.*, 1939), and also from spleen and parasite rates in adults (Gilroy, 1939; Russell and Jacob, 1942, Covell, 1939). Dakshinamurthy (1948) described a statistical technique for measuring malarial incidence.

Malaria mortality . In studying the fluctuations of malarial mortality 'epidemic figures' are used (Yacob and Swaroop, 1945).

ADULT MOSQUITOES

A minimus The reactions of *A. minimus* to light during sunset and sunrise have been studied Their daytime resting places, and mating, blood feeding, and ovipositing habits have been noted There is no evidence to show that *A. minimus* hibernate in cold season (Thomson, 1941*c*).

A culicifacies The daytime resting place, range of flight and biting, ovipositing and mating habits and other behaviours of *A. culicifacies* have been investigated (Afridi and Puri, 1940, White, 1940*b*; Russell and Rao, 1942*a*, Russell *et al.*, 1944, Pal, Rajindar, 1943*a*). *A. culicifacies* does not appear to undergo hibernation The time when this mosquito begins to enter houses and the hourly variations in their numbers was recorded by White (1946*b*).

A sundaicus and *A. subpictus* The swarming and pairing habits of these two species of mosquitoes have been recorded by Rao *et al* (1942). *A. subpictus* is not exclusively zoophilic. When conditions are favourable it will like to feed on human beings also (Roy, 1943).

A philippinensis It prefers to take shelter in the dark corners of houses, rather than in cattlesheds (Ganguli, 1947).

A fluviatilis · The habits and behaviour of *A. fluviatilis* such as house entry, breeding etc, were investigated by several workers (Russell and Jacob, 1942; Viswanathan and Rao, 1943, Viswanathan *et al*, 1944; Rao, T R., 1945, White 1945). The habits of other species belonging to *fluviatilis* group was studied by White (1941). Mohan (1945) described a method for rearing a colony of *A. fluviatilis* in the laboratory.

A. stephensi . It is very easy to rear in cages the type form of *A stephensi* but not var *mysorensis* This is because the females of var *mysorensis* fail to lay eggs in the cages (Russell and Mohan, 1941*a*)

A. superpictus It is a large mosquito with longer range of flight than either *A. culicifacies* or *A stephensi* (De Burca, 1939).

Although the females of some anopheles mosquitoes such as, *A. culicifacies* and *A. stephensi* would feed on blood of animals, when given the choice they will prefer human blood. There are other species whose females feed exclusively on animal blood (zoophilism) Food preference of mosquitoes may be determined by precipitin tests (Afridi *et al.*, 1939). Roy and Ganguli (1943) have described the method of preparing precipitating sera and performing precipitin tests. Mosquitoes fond of human blood show higher anthropophilic indices than those which feed on animal blood (White, 1945)

There is a relation between digestion of blood and development of ovaries. Under experimental conditions most anopheles mosquitoes complete the *gonotropic cycle* within 48 hours during summer (White, 1945). But during autumn certain degree of gonotropic dissociation may take place among some mosquitoes, *although there is no general suspension of their sexual activities.* Under such conditions the development of ovaries does not keep pace with nutrition (Rao, V. V., 1947) During the gonotropic cycle the mosquitoes generally remain indoor, but sometimes a dangerously high proportion of these mosquitoes may leave houses before digestion is complete (White, 1945*a*; White, 1946*b*).

The longevity of anopheles mosquitoes depends on sex and species. Thus while the maximum longevity of *A. culicifacies* females is between 8-34 days (Russell and Rao, 1942*b*), in the case of *A minimus* and *A. jeyporiensis* this is, however, about 12 days (White, 1945). The longevity is also influenced by atmospheric temperature and humidity (Knowles and Basu, 1943, Pal, Rajindar, 1943*a*; Siddons, 1944*b*).

The susceptibility of mosquitoes to infection varies with the species of malarial parasite Thus while *A annularis, A. subpictus, A. aconitus, A. culicifacies, A barbirostris* and *A. hyrcanus* are susceptible to infection with *P. cynomolgi* (Rep. Scient. Adv. Board, IRFA, 1946), *A. stephensi* and *A annularis* are so with *P knowlesi*. In India *A maculatus* is highly refractory to the local strains of malarial parasites (Strickland *et al*, 1940). The susceptibility of mosquitoes to infection depends on several factors, such as : (a) atmospheric temperature and humidity (Knowles and Basu, 1943; Basu, 1943), (b) host-parasite specificity (Russell and Menon, 1942), (c) virulence of the parasites (Strickland *et al.*, 1940). According to Singh *et al.* (1949*a*, *b*) the virulence does not increase on passage through susceptible mosquitoes, and (d) viability of the gametocytes. This is altered in presence of gametocidal drugs in the blood. Prontosil has no gametocidal action (Chopra and Basu, 1939*a*). Although paludrine has apparently no action on the gametocytes, yet the gametocytes from persons receiving paludrine fails to develop in the mosquito host (Chaudhuri and Rai Chaudhuri, 1949*a*). In this connection it may be said that the chemical composition of the water of the breeding place does not alter the susceptibility of the adult mosquitoes (Russell and Mohan, 1939*a*, *b*, *c* and 1941 *b*).

When experiments are designed to test the susceptibility of mosquitoes, insectary bred *A. stephensi* may be used as control (Russell and Mohan, 1939*b*, *c*). The two biological races of *A. stephensi* are equally susceptible to *P falciparum* (Russell and Mohan, 1939*b*, *c*).

Regarding the visual response of the mosquitoes, it has been observed that during the day time the anopheles mosquitoes avoid light but from the onset of dusk they are attracted by the former (Rao, T.R., 1947).

Mosquitoes may be dispersed by railway trains, country boats, etc. (Sen, 1941*a*, *b*).

Biological races: Biological races exist within the same species of mosquitoes. Proofs on this have been obtained (Rep Mal Inst, India for the year 1939; Pal, Rajindar, 1943*b*; Vankat Rao, 1949). White (1947 and 1948) is of opinion *that not only in mosquitoes but also in other insects there are separate races adapted* to different food and modes of life.

Catching of mosquitoes: When this is required for species and sex determination hand catching may be omitted, but when the object is to check the effects of insecticidal sprays hand catching and search of floors for dead specimens are to be *carried out simultaneously* (*White and Rao, 1946b*)

Larvae

The larvae of many species of anopheles mosquitoes are similar in their habits This is most marked between *A culicifacies* and *A subpictus*, *A hyrcanus* and *A. pallidus*, *A annularis* and *A jamesi*, *A. stephensi* is the exclusive species (Russell and Rao, 1940*a*) During winter the morphology of the larvae of *A. fluviatilis* may change This has been noticed by De Burca and Forshaw (1947)

When identification of the full grown larvae of the Indian anopheles mosquitoes is required the synoptic table prepared by Puri may be consulted Sen (1949) *described the diagnostic characters of the larvae of A subpictus and A. sundaicus.*

There are six species of larvae which are liable to microsporidial infection (Sen, 1941*b*).

Regarding the density of larvae Russell *et al* (1945) computed various expressions by simple and partial correlation technique.

Eggs

The eggs of *A. varuna* at Vizagapatam are shorter than those at Jeypore Hills (White and Rao, 1943).

Malarial Parasites

The sporozoites of *P. gallinaceum* are unable to pass through the skin even when scarified (Rep. King Inst., Guindy, 1940-41). Within an hour after injection the sporozoites of *P. cynomolgi* disappear from the skin. They circulate in the blood for 45 min, thereafter enter internal organs to take up exo-erythrocytic phase of life cycle. No developmental forms of *P. cynomolgi* have been detected at the site of inoculation (Rep. Scient. Adv. Board, IRFA, 1946, Mulligan *et al.*, 1949). Bhatt (1949) detected sporozoites of doubtful origin in the salivary glands of a specimen of *A. turkhudi*.

The sporozoites of *P gallinaceum* remain viable in normal and 2 per cent citrated salines for 1½ hours. When infected *aedes* mosquitoes or the site inoculated with sporozoites of *P. gallinaceum* are exposed to X-rays, such exposures do not alter the viability and the infectivity of the sporozoites The infectivity is at its maximum at 72°–82°F (Rep King Inst., Guindy, 1940–41, Rep. Scient. Adv. Board, IRFA, 1946).

In the internal organs the sporozoites develop into exo-erythrocytic schizonts. In case of *P gallinaceum* the E E schizonts develop in the endothelial cells of brain capillaries of fowls within 5 days after inoculation (Rep King Inst., Guindy, 1940-41, Setharam Iyer *et al.*, 1941; Shortt and Menon, 1941). Attempts to demonstrate E E. forms simulating those of avian malarial parasites in infected monkeys and squirrels have failed (Rep. Scient. Adv. Board, IRFA, 1946).

The erythrocytic forms appear in the peripheral blood after an incubation period which varies with species of parasite In the case of *P. cynomolgi* this is 15 days (Rep Scient. Adv. Board, IRFA, 1946). Under certain circumstances the *erythrocytic forms* of *P vivax* may show some abnormalities such as 12 merozoites in the schizonts, and fimbriation of infected R.B.C.'s (Das Gupta, 1939*a*). Mulligan and Sommerville (1947) have studied the morphology, pathogenicity and infectivity of *P. vassali* var *ratufae*. This is a new parasite which has been discovered by Mulligan and Sommerville (1947) from a Malabar squirrel.

On the life history of *P falciparum* certain important observations have been made by Basu (1939) in relation to the crescents. Das Gupta and Ganguli (1944) have shown that in fatal *P. falciparum* infections developing schizonts and gametocytes appear in peripheral circulation shortly before death.

Regarding the infectivity of the trophozoites it has been observed that exposure to X-ray of the site inoculated with infected blood, does not alter the infectivity of the parasite. Sunlight and methylene blue, on the other hand, produce opposite effects. After being mixed with 1 : 50,000 solution of methylene blue if the infected blood is exposed to sunlight for 5 min, the trophozoites lose infectivity (Rep. King Inst., Guindy, 1940-41).

Malarial infections occur naturally from the bite of infected mosquitoes. The infection may be produced artificially by injection of blood containing trophozoites Malaria sometimes occurs following therapeutic transfusion of blood if the donor's blood happens to contain parasites (Das Gupta, 1943). Besides inoculation per oral administration of infected blood may also give rise to the malaria. This has been demonstrated by Shortt and Menon (1940*a*, *b*) in case of rhesus monkeys and fowls.

Malarial parasites may pass from the mother to the foetus through the placenta only if the placenta is injured as a result of a chronic infection (Das Gupta, 1939). In acute infections, however, transplacenta passage of the parasites does not occur (Strickland and Baird, 1939)

Regarding the gametocytes, it may be said that a typical gametocytes may sometimes appear in peripheral circulation This has been reported by Noronha (1945). In the gut of the mosquito the male and female gametocytes produce the male and female gametes respectively, which by their union lead to the formation of oocysts The oocysts may show abnormalities such as formation of black spore, twin oocyst etc (Basu, 1947).

Cultivation of malarial parasites Niyogi and Roy (1942) cultivated *P knowlesi* parasites according to Bass and John's method When such cultures are kept at 24°C the trophozoites retain infectivity for 4 days, but when incubated at 37°C, the infectivity is lost within 2 days

Reservoir hosts of malarial parasites · It is suspected that the original host of *P. gallinaceum* is the jungle fowl from which the infection is transmitted to the domestic fowls (Shortt *et al*, 1941) Natural host of *P. inui* is probably *S. sinicus* monkey (Mulligan, Somerville and Swaminath, 1940*e*)

Pathology

In cerebral malaria the capillary endothelium is damaged by malarial toxin This together with the slowing of circulation caused by the fall of blood pressure facilitates formation of thrombus The thrombus formation may occur in saggital sinus also (Viswanathan, 1944).

Anaemia is common in malaria. Majumdar and Das Gupta (1944) showed that in acute *P. knowlesi* infection in rhesus monkeys anaemia develops steadily as the parasite density exceeds 10 per cent. In subacute and chronic infections, however, anaemia develops very slowly.

Immunity

In the defence against malarial infection three factors are involved, these are: an inherent quality of the host (natural resistance) checking the development of

the parasites (Mulligan *et al.*, 1940*c*, *d*), a cellulo-humoral factor responsible for acquired active immunity (Sinton 1939*a*, *b*; Mulligan *et al.*, 1940*b*; Dikshit, 1941), and an antitoxic element neutralising the hypothetical toxin. (Sinton, 1939*a*, *b*).

The cellular factor is the phagocytic activity of the lymphoid macrophage system. The specific humoral factor is probably of the nature of opsonin. Active immunity is species-specific and as such cannot check or modify the course of infection due to other species (Singh and Singh, 1940*b*). McGuire (1944) and Sinton 1939*a*, *b*) have produced evidences establishing the existence of active immunity in malaria.

Injections of vaccines prepared from killed trophozoites do not confer protection against infection by homologous strain of the parasite although Niyogi (1942*a*) states the contrary. The immune reactions, however, are strong if the vaccines (prepared from sporozoites) and immune sera are injected together (Shortt and Menon, 1940*a*, Russell and Mohan, 1942).

Diet has no effect on the course of experimental malaria in monkeys (Passmore and Sommerville, 1940) The duration of immunity varies with the species of parasites causing infection (Sinton, 1939*a*, *b*; Singh and Singh, 1940*b*).

The immunity may be transferred passively by injecting relatively large quantities of immune serum (Singh and Singh, 1940*c*) Injections of a saline extract of malarial spleen, likewise offers protection against malarial infections. A greater degree of protection is acquired if prior to injection of the saline extract (or the immune serum), the lymphoid-macrophage system is stimulated (Mulligan *et al.*, 1940*c*).

Agglutinins and complement-fixing antibodies develop in the sera of animals and persons suffering from malaria or injected with killed parasites Agglutinins appear after the acute phase of infection has passed off. The titre rises as the infection becomes chronic and after each super-infection. Presence of agglutinins in the serum may be demonstrated by agglutination of R.B.C.s infected with homologous parasites. The reaction is specific (Singh and Singh, 1940*a*). Ray *et al.* (1941*a*) have shown that following the injection of killed parasites agglutinins develop only occasionally.

It has been observed that the sporozoites of *P. gallinaceum* agglutinate not only in presence of (i) sera from fowls chronically suffering from the infection, but also in presence of (ii) normal sera from man and animal and (iii) sera of animals which have suffered from malaria. In the case of (i) agglutination occurs in high dilutions. Sporozoital agglutination is a specific reaction (Mulligan and Russell, 1940; Mulligan *et al.*, 1940*a*). Injections of sporozoites inactivated by X-ray radiation or emulsions of dried and ground-up thoraces of infected mosquitoes also lead to the development of specific agglutinins in high titres (Mulligan *et al.*, 1941; Russell *et al.*, 1941).

Complement-fixing antibodies develop in the sera of rabbits after the inoculation of killed malarial parasites (Ray *et al.*, 1941*b*). Complement-fixation reaction is group-specific, and as such *P. Knowlesi* antigens give positive reactions with the sera of persons suffering from *P. vivax* infection (Niyogi, 1942*b*). In fatal infections the complement content of the serum rapidly diminishes during later part of infection (Roy and Mukerjee, 1942).

In the defence against malarial infection spleen plays a very important part This has been demonstrated by several workers (Mulligan *et al.*, 1940*c*; Lowe, 1945; Rep. Sci. Adv. Board, IRFA, 1946). If malaria is contacted in the absence of spleen, the liver will take up the functions of the former and in so doing will undergo compensatory hyperplasia (Lowe, 1945) In fowls the spleen is enlarged also after the injections of sheep serum, inactivated sporozoites and infected blood. The enlargement also occurs when the fowls suffer from *P. gallinaceum* infection and this becomes greatest when the infection has become chronic and the fowl has received vaccines and immune sera at the same time (Russell *et al.*, 1943*a*).

CLINICAL

In malaria the cerebrum is affected principally by *P. falciparum*, although cases of cerebral malaria due to *P vivax* infection have been reported (Mohapatra, 1948) Manifestations of cerebral malaria reported by various authors are. (i) coma, aphasia, hemiplegia and fever (Roy, 1939, Still and Lal, 1941; Gupta and Laha, 1944), (ii) coma and fever (Dhayagude and Purandare, 1943); (iii) unconsciousness, epileptiform convulsions and sometimes paralysis of the soft palate (Bhattacharjee, 1941; Nandi, 1949); (iv) fever and psychosis (Sarkar, 1941*b*) and (v) symptoms of meningitis (Narayan, 1941). In cerebral malaria presence of sugar or albumin in urine, absence of splenic enlargement and high temperature (in such cases the temperature is usually fixed round 101°F) indicate fatal termination (Hamburger, 1944).

Malarial parasites are responsible for pathological changes in other viscera also. Thus in the case of heart they produce heart block (Nandi, 1949) or acute cardiac failure (Sarkar, 1941*a*). Involvement of alimentary tract, on the other hand, leads to conditions simulating, acute abdomen (Rao, T M, 1948; Nandi, 1949), abdominal colic (Mukherjee and Mitra, 1950), diarrhoea (Chaudhuri and Chakravarty, 1950), cholera, acute bacillary dysentery (Pattanayak, 1940) and melaena (Roy, 1939). Heilig and Sharma (1948) have reported a case of pneumonia due to malaria.

Besides the above malaria may also be responsible for allergic manifestations such as, urticarial rash, joint pains, etc. (Chatterjee, 1939, Mohapatra, 1949) and other conditions namely, sciatica (Wahi, 1950), dendritic keratitis (Falcone,

1946), acute lymphangites (Nandi, 1949), internal haemorrhage due to rupture of enlarged spleen (Stonham, 1945; Bilangady, 1947), Raynaud's phenomenon (Chatterjee, 1941), paroxysmal haematuria (Dutt, 1942) and nephritis (Laha, 1945).

Infantile malaria : Malaria in infants is very common in hyperendemic localities. Several cases have been recorded where malaria occurred in infants within 15 hours, 5 days and 7 days after birth (Das Gupta, 1939*a*; Strickland and Baird, 1939).

Malaria due to P. ovale : Malaria due to *P. ovale* infection occurs in India (Raman, 1940).

DIAGNOSIS

Thick blood films when properly stained give the most reliable information (Lowe, 1944). For staining thick films, as an alternative to the usual Romanowsky's stain, Jaswant Singh's modification of Field's quick method of staining (Rep. Ross Inst. Trop Hyg., India Branch, 1947) or the method of Singh and Bhattacharji (1944) may be employed. The method of staining malarial parasites as advocated by Boye is very simple and reliable and thus it may be tried (Simeons, 1942). When blood films are negative, fresh examinations may be made three or four times thereafter, if necessary, after an injection of adrenaline (Hunter, 1945). Sternal puncture does not give more information than does a thick blood film (Lowe, 1944) Moreoever, sternal and spleen punctures are not suitable for routine examinations (Hunter, 1945).

The serological method for diagnosis of malaria include buffer precipitation test (B.P.T.), Weltmann coagulation reactions (W C.R.) and serum flocculation test Wolff (1940) has described the technique of B.P.T., while Singh *et al.* (1950*b*) have performed W.C.R. using sera from normal as well as infected monkeys It has been demonstrated by Naidu *et al.* (1942) that addition of ethyl alcohol to the distilled water increases the sensitivity of serum flocculation test.

Staining of Oöcysts and Sporozoites · Oöcysts in the midgut of mosquitoes may be stained with 0.1 per cent aqueous solution of toluedine blue, undiluted Leishman stain, Giemsa stain or J.S.B stain after fixing the gut in 10 per cent formol saline. Sporozoites may be stained by J.S B. technique (Singh and David, 1949).

TREATMENT

Quinine Intravenous quinine is of greatest value during emergencies (Covell, 1943). In choleraic malaria the quinine solution for the sake of convenience may be injected into the rubber tubing of the transfusion set at the time of saline transfusion (De, 1939). Intramuscular injections are recommended only when oral or intravenous administrations are not feasible (Lowe, 1944; Dauncey, 1941).

Chakravarty and Basu (1950) claimed that in preparing quinine tablets if dehydrocholic acid be combined with quinine sulphate, the former will increase the therapeutic efficacy of the latter. In absence of quinine bihydrochloride solution, quinine sulphate may be injected after dissolving it in acidified distilled water and boiling (McGuire, 1944).

Although it is said that administration of a single dose of quinine leads to the disappearance of parasites from the peripheral circulation, this is not always true (Bardhan, 1941) Quinine preparations available in Indian market did not conform to the standards laid down (Bose *et al.*, 1939).

Some of the toxic symptoms of quinine reported were myocardial impairment (Heilig and Visveswar, 1944) and amblyopia (Guha, 1946, Das Gupta, 1949).

Several attempts have been made to prepare anti-malarial drugs related to mepacrine (Guha and Mukherji, 1946) But the one having the chemical formula, 2-chloro-7-methoxy-5 (8-diethyl-amino-butyl), amino-acridine was found to be effective against both human and simian malarias (Siddons and Bose, 1944, Bose and Rakshit, 1944).

After considering the therapeutic effectiveness of mepacrine, Rogan and Coombes (1945) advised the withdrawal of quinine and pamaquine from the schedule of Army standard treatment and insertion of mepacrine in their place.

Some of the toxic symptoms of mepacrine reported were maniacal excitement (Berry, 1945, Rao, 1947*a*) and temporary mental disorder with intense sexual excitement (Kapur and Das Gupta, 1950).

Plasmochin · Although plasmochin is a reputed gametocidal drug, its chief function is reductions of relapses (Covell, 1943) It does not filter through the placenta in doses sufficient to affect the respiration or heart's action of the foetus (Dikshit, 1939).

Paludrine Clinical studies indicated that paludrine was one of the effective anti-malarial drugs (Das Gupta, *et al*, 1945; De and Datta, 1947*a*, Ghosh and Ghosh, 1947, Sen, 1947, Lahiri, 1947, Ansari, 1948) Various dose-schedules were recommended, but a single dose of 300 mg appeared to give good result (Chaudhuri and Rai Chaudhuri, 1947, Parekh and Beghani, 1947*a*, Viswanathan and Baily, 1947; Srivastava, 1947, Jafar, 1947, Ghosh, 1947, De and Dutta, 1947*a*; Parekh and Beghani, 1947; Lemax, 1947; Afridi, 1947). During emergencies paludrine may be administered intravenously in the form of paludrine acetate solution or a solution prepared by dissolving the tablet in distilled water may be used (Chaudhuri and Chakravarti, 1949; Mullick and Gupta, 1947 and 1948). However, in extreme emergencies paludrine should not be used as its action is not so rapid and dependable as that of quinine (Chaudhuri and Chakravarti, 1950).

Apart from the curative value paludrine was also found useful as a casual prophylactic (Mullick, 1948; Ray, 1948).

Although the drug is active against all types of malarial parasites its action is more rapid against *P. falciparum* than *P. vivax* (Chaudhuri and Rai Chaudhuri, 1947). In India some strains of *P. falciparum* have been found to be resistant to the action of paludrine (Chaudhuri and Rai Chaudhuri, 1949*a*).

After paludrine therapy relapses are more common in B.T. than M.T. infections (Parekh and Beghani, 1947). Relapses are rare with higher doses (Chaudhuri and Rai Chaudhuri, 1947).

Paludrine did not produce any notable toxic reactions (Chaudhuri and Rai Chaudhuri, 1947) although some persons may suffer from idiosyncracy (Parekh and Beghani, 1947) Toxic reactions were negligible after intravenous injections were recommended, but a single dose of 300 mg appeared to give good result (Mullick and Gupta, 1947 and 1948, Chaudhuri and Chakravarti, 1949).

Chloroquine It appears to be a better drug than quinine, mepacrine and paludrine It is effective against the asexual forms of malarial parasites Relapses are common. Toxic reactions are negligible It may be given as a single or in divided doses (Chaudhuri *et al*, 1948, Goldsmith, 1946)

Camoquin It is as effective as chloroquine Relapses are common It has no toxicity It may be given in divided doses, but single dose treatment gives the best result (Patel and Mehta, 1948, Chaudhuri and Chakravarti, 1948; Simons and Chhatre, 1947).

Amongst atebrin, paludrine, resochin, camoquin, metachloridine and aphacrine, camoquin and chloroquine act most rapidly in simian malaria. It is claimed that atebrin, paludrine and chloroquine cause radical cure. Camoquin seems to be the most effective drug (Singh *et al*., 1949). However, for single dose treatment in rural areas chloroquine is most suitable (Chaudhuri *et al*., 1950).

Sulpha-group of drugs : Sulpha-group of drugs vary in their reactions against human and simian malaria parasites White M. & B 693 and thalazole derivatives are effective when given in heavy doses, others such as prontosil are without effect (Chopra and Das Gupta, 1939, Chopra *et al*., 1939; Singh and Singh, 1939; Dikshit and Ganapati, 1940; Patel, 1944). Sulpha-drugs have no action on gametocytes (Chopra and Basu, 1939*a*, *b*).

Other drugs : Some drugs claiming antimalarial properties have been investigated. These were : (i) indigenous drugs such as, *Alastonia scholaris*, *C. benducella*, *Fraxinus melacophylla* and *E. littlerale* (Mukherji *et al*., 1942 and 1943; Mukherji, 1946, Das Gupta *et al*., 1944; Rai, 1946), (ii) organic arsenicals (Das Gupta and Siddons, 1944), (iii) stilbamidine (Das Gupta and Siddons,

1944), (iv) acridine derivatives (Patel *et al*, 1948, Kshatryya *et al*, 1950), (v) M_3 (Chopra *et al.*, 1940*a*), and (vi) neochin (Singh, 1949; Chaudhuri and Rai Chaudhuri, 1949*b*). Among the substances prepared after structural formula of paludrine, bromoquinidine has been found useful to some extent (Singh *et al*, 1949*a*).

Deshmukh (1947) has demonstrated that combination of penicillin with specific anti-malarial drugs does not serve any useful purpose Berberin sulphate was suggested as an adjuvant to quinine as the former might drive away the parasites from internal organs to be killed by the latter (Brahmachari, 1944)

In treating a case of malaria whether the treatment should aim at 'radical cure, clinical cure or clinical prophylaxis will have to be judged from whether the person is exposed to frequent and constant risk of infection, re-infection or super-infection (Sinton, 1939*a*, *b*).

Malaria therapy · In the treatment of neurosyphilis, inoculation of blood *containing P. falciparum is preferred. As mixed B T. and M T infections* are common in India, after the infective inoculation daily blood examinations are essential (Chopra *et al.*, 1941*a*, *b*).

Pharmacology of anti-malarial drugs · Anti-malarial drugs may sometimes fail to act due to delayed absorption from the intestine (Chopra and Chopra, 1945). Wahi (1947) investigated the effects of some anti-malarial drugs on plasma prothrombin level Nandi (1940) showed that oxygen uptake by different tissues of a guinea-pig increases in the presence of plasmochin

Preparation of anti-malarial drugs · Several attempts were made to prepare anti-malarial drugs (Bami *et al*, 1946, 1947*a*, *b*, 1948*a*, *b*, *c*, 1949*a*, *b*).

MALARIA CONTROL

It includes, antilarval measures, control of adult mosquitoes, suppressive drugs treatment and combination of the above three.

Antilarval measures The larvae of anopheles mosquitoes may be eliminated by preventing breeding of mosquitoes (naturalistic control) or directly by killing the larvae by biological and chemical means.

Breeding may be prevented by (a) periodic flushing of streams and drains by building dams and sluices across the stream or by siphoni (Banerjea, Covell and Harbhagwan, 1939; McDonald, 1939; Ramsay and Anderson, 1947); (b) herbage packing of rice fields and drains (Covell and 1939; Nagendra, 1947); (c) shading water surfaces with Eichhornia (Iyengar, 1946; Rao and Ramakrishna, 1947) or by growing creepers and perineal shrubs on the banks of canal

Ramee, 1942, Thomson, 1942*b*); (d) deweeding, clean edging and training of streams (Covell and Harbhagwan, 1939, Mondal, 1940; Covell and Singh, 1942, Subraraman and Vedamanikkam, 1943, Rao and Ramakrishna, 1947); (e) removal of vegetations from water surface and exposure of the latter to sunlight (Thomson, 1942*b*), (f) filling draining and tidying of channels (Ahmed, 1942, Knipe and Russell, 1942*b*), (h) intermittent irrigation (Knipe and Russell, 1942*b*; Russell and Rao, 1940*a*, *b*, Russell *et al.*, 1942*b*); and (i) removing pistia (Roy and Roy, 1941, Covell, 1947).

But Russell and Jacob (1939*b*) stated that when they applied naturalistic methods to 'casuarina pits' for control of anopheline breeding none gave satisfactory results.

In biological control of the larvae small fishes such as *Gambusia affinis*, *Aplocheilus lineatus*, *Panchex parvus* and *Discognathichthys rossicus* var *nudiventris* are stocked in wells and tanks These fishes eliminate the larvae by feeding on the latter (Russell and Jacob 1939*c* John, 1940, Rao and Ramee, 1942; De Burca, 1939) Masillamani (1946*b*) described a net for catching *Gambusia* fish from nurseries and tanks

The larvae of anopheles mosquitoes may be killed by oils (Misra, 1941), Paris green, active principles of certain plants, and other chemical substances. Paris green is a good larvicide (Rao, 1945; Rao and Nassiruddin, 1945). It may be applied mixed with sand or as an emulsion (Russell and Jacob, 1939*b*; Russell *et al*, 1940) Paris green may be distributed with the help of automatic distributing machines (Knipe and Russell, 1942*a*, Russell *et al*, 1940, Massillamani, 1946*a*). In quantities in which it acts as larvicide it does not harm rice plants (Sen, 1939).

The plants such as Duranta, Zanthoxylum, Gardenia and Tephorosia contain principles which are active against the larvae of mosquitoes (Mansen, 1939; Chopra *et al*, 1940*d*, *e*, *f*) The essential oils of *Atremisia vulgaris*, *Oscimum basilieum* and *O sanctum* are larvicidal as well as insecticidal (Chopra *et al.*, 1940*f*; Chopra *et al*, 1941*b*) *Blumea densiflora*, however, does not contain any larvicidal principle (Chopra *et al.*, 1940*f*).

The other chemicals active against the larvae are · (i) a mixture of diesel oil and cresol (Covell and Afridi, 1939), (ii) solid residue left after kerosene extraction of the active principles of pyrethrum (Chopra *et al.*, 1940*e*), (iii) DDT as solution in kerosene or emulsion in turpentine (Puri and Pal, Rajindar, 1947; Nagendra and Murphy, 1949); (iv) 5 per cent DDT in malarial and 2 per cent Gammexane liquid concentrate LG 140 (Bertram, 1950); and (v) 0.75 per cent ammonium sulphate (Brink and Das Chowdhury, 1939). Russell and Rao (1941) showed that lowering of the surface tension of water due to addition of soap causes the larvae to sink and die.

White and Narayan (1940) showed that antilarval measures when directed against different species of mosquitoes, did not give uniform results. However, during the cold season the cold water breeding places, where these exist, should be dealt with (Thompson, 1941*c*) and in case of *A. fluviatilis* the antilarval measures should be extended up to at least 1000 feet to ½ mile from the nearest human habitation (Adisubramaniam and Vedamanikkam, 1943; Rao and Philip, 1947)

Control of adult mosquitoes In the control of adult mosquitoes repellents are not very useful. Philip *et al* (1945) studied the repellent actions of tumeric and of some vegetable oils Insecticides unlike the repellent knock down the adult mosquitoes. As insecticides, pyrethrum, DDT and Gammexane have gained reputation

Pyrethrum It may be sprayed as a mixture in kerosene (Russell and Knipe, 1939, 1940) or as an emulsion in water (Russell *et al*, 1942*c*). Extracts of Indian pyrethrum flowers are as effective as the foreign makes (Russell and Knipe, 1941, Russell *et al*, 1943*b*) It is highly active against adult mosquitoes (Covell and Afridi, 1939, De Burca, 1939, Rep Mal Inst, India for 1939 and Viswanathan, 1941*a*, White, 1945)

DDT · Its usefulness in the control of anopheline mosquitoes has been established by several workers (Puri and Pal, Rajindar, 1947, Rao, 1948, Hajra, 1948, Viswanathan and Rao, 1948 and 1949, Venkat Rao, 1949, Singh and Kariapa, 1949, Vedamanikkam, 1949, Subramanian *et al*, 1949, Nagendra and Murphy, 1949). DDT may be applied as an indoor residual spray, outdoor spray or as a space spray, either in the form of a solution in kerosene, malarial, used engine oil, as an emulsion in kerosene or turpentine or as a suspension (Puri and Krishnaswami, 1947; Puri and Bhatia, 1947, Afridi and Bhatia, 1947, Puri and Pal, Rajindar, 1947; Viswanathan and Rao, 1947, Afridi and Singh, 1947; Vedamanikkam, 1949). While spraying surfaces made of straw or palm leaf suspensions of DDT have been found to be more useful than the solutions or emulsions (Sundara Raman and Peffly, 1949).

DDT reduces the infectivity as well as the density of the adult mosquitoes (White, 1945, White and Ghosh, 1946) Different species of mosquitoes show different degrees of susceptibility to DDT (White and Ghosh, 1946; Viswanathan and Parekh, 1946) The residual toxic action of DDT lasts from 3 to 5 months (Ramakrishnan *et al*, 1948)

Gammexane (*BHC*) Both DDT and Gammexane control malaria. Although previously it was claimed that gammexane was superior to DDT (Rep Scient. Avt. Board, IRFA, 1948), now it has been shown that the things are the opposite (Rep Scient. Adv. Board, IRFA, 1949; Viswanathan *et al.*, 1949; McDonald, 1950).

Sprayers and methods of spraying : For spraying the insecticides, different types of sprayers such as, hand atomiser, hand pump air tank, power-filled air tank, power-operated 'two man' and 'one man' pressure outfits etc. have been invented (Wats and Bharucha, 1940, Russell and Knipe, 1941; Knipe and Sitapathy, 1942; Covell and Singh, 1943, Russell *et al.*, 1943*b*). Spraying may be done daily or intermittently (White and Rao, 1944; Viswanathan *et al.*, 1944; White, 1945).

Spray killing of adult mosquitoes is the only measure which produces an immediate effect on an epidemic of malaria. It involves an initial outlay but in the long run the maintenance cost will be much cheaper than the cost it would require for the continuance of antilarval measures (Covell, 1942*a*).

Suppressive drug treatment : For prophylaxis of malaria mepacrine and quinine are useless (Chopra and Basu, 1939*a*). Although atebrin is effective in suppressing clinical attacks of malaria, paludrine, chloroquine and camoquin have been found to be far better than the former (Lamprell, 1940, Hunter, 1945, Goldsmith, 1946; Lemax, 1947, Banerjee, 1949; Rep. Scient Adv. Board, IRFA, 1949; Adhikari, 1949).

Singh *et al.* (1950*a*) studied the suppressive actions of chloroquine, camoquin, mepacrine, aphacrine, and proguanil (paludrine) against blood-induced as well as sporozoite-induced *P knowlesi* infections in rhesus monkeys.

Combination of different control measures : DDT spray may be combined either with antilarval measures or with suppressive drug treatment (Singh and Singh, 1949; Russell *et al*, 1943*b*; Adhikari and Ganguli, 1949). According to Viswanathan and Parekh (1946) combination of different methods is always more effective than a single one Rao (1949) made a critical review on the methods of control of malaria in India.

Personal protection. In preventing malaria personal protection against the bite of mosquitoes has some importance. Covell (1943) considered that shorts are entirely unsuitable for campaigning in tropics.

The benefit which the various control measures produce after their execution may be estimated from decline of spleen and parasite rates (Ahmed, 1939; Afridi and Bhatia, 1947) and also from vital statistic (Viswanathan, 1949).

Economic aspect of malaria. The direct financial loss that a rural community in India suffers due to malaria is considerably more than the amount it would cost to control the disease (Russell and Menon, 1942)

The progress of malaria control in India up to 1941 was satisfactory but not up to expectation. This was due to some social obstacles (Watson, 1940; Russell, 1941).

HAEMOPROTEUS COLUMBAE

Bose and Rakshit (1944) investigated into the activities of certain substituted quinoline and acridine compounds against the gametocytes of naturally acquired *Haemoproteus columbae* infections in pigeons

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LEISHMANIASIS

KALA-AZAR

Epidemiology :

Kala-azar is confined to the eastern half of Indian peninsula comprising Assam, Bengal, Bihar, Orissa and the eastern half of U P as far west as Lucknow (Bhende *et al.*, 1949). Although, from time to time reports on the existence of indigenous kala-azar in the western half of the country appear, these are, in fact, cases imported from endemic foci (Raman, 1944, Row and Patkar, 1947; Heilig and Sachdev, 1947, Chand *et al*, 1949, Raghavan, P 1949, Bhende *et al*, 1949; Bhende, 1950).

The distribution of kala-azar in Bengal is very irregular The incidence is high in the district of Murshidabad, Malda, Rajshahi and Jessore but low in Bankura and Birbhum (Sen Gupta, 1944*f*) The irregularities in the incidences are also noticeable in the city of Calcutta. While the centre of the city is remarkably free from the disease, it is endemic in its other parts (Sen Gupta, 1947*b*).

There was a sharp rise of kala-azar in Calcutta in the years following Bengal famine in 1943 (Sen Gupta, 1947*b*). At about the same time (1942) an epidemic started in U P and this continued up to 1947. Previous to 1942 kala-azar in U.P. was, however, very low (Prasad, 1949).

The factors responsible for epidemics of kala-azar directly or indirectly, have been described by Sen Gupta (1947*b*) and Prasad (1949).

Transmission :

After a blood meal if the sand flies are kept on raisins instead of repeated blood meals several things happen, viz, (i) the flies live longer than the usual 10 days, (ii) a larger portion of the flies get infected, and (iii) an intense development of the flagellates "blocks" the infected flies. Blocking is detected after 10 days when a second blood meal is offered. The blocked flies make frantic efforts for sucking blood The struggle for blood dislodges the flagellates at the site of bite wound (Smith *et al*, 1940*b* and 1941*a*, *b*). Only the flies kept on raisins are blocked and not those maintained by repeated blood meals (Smith *et al.*, 1941).

Using blocked flies attempts were made to transmit kala-azar into animals and human volunteers (Smith *et al*, 1940*a*, *b*, Swaminathan *et al*, 1942). The results were highly satisfactory. According to Smith *et al.* (1941*a*, *b*) the success in these experiments did not accrue from an increased virulence of the particular parasite used, but resulted from the special method of feeding the flies

Regarding the longevity of the blocked flies, Smith *et al.* (1941*a*) showed that

majority of the flies died within 24 to 72 hours after the block, although a few may survive as long as the 6th day.

It was suggested by Napier and Krishnan that kala-azar might result from dissemination of the parasites from a quiescent focus in the skin following an attack of malaria or typhoid To study this point Smith and Ahmed (1941) made some field experiments in Bihar and showed that Napier and Krishnan's *suggestion* was without foundation

Clinical :

The two symptoms which are constantly present in kala-azar are (i) irregularly remittent types of temperature, and (ii) rapid pulse The other symptoms such as, marked toxaemia, headache, pains and aches in the body and the limbs are absent in kala-azar (Lowe, 1944) Delirium is not a common symptom, but it may sometimes appear (Bhaskaran, 1949) Hardy and Passmore (1943) discussed certain features of kala-azar cases as seen in India

Complications

The noteworthy complications of kala-azar are agranulocytosis, cancerum oris and pneumonia *Acute agranulocytosis is rare in India The oropharyngeal symp*toms of agranulocytosis may sometimes be absent or inconspicuous (Das Gupta and Sen Gupta, 1943 Sen Gupta and Chakravarty, 1947) Banerjee (1950) has described a case of severe granulopoenia which was associated with sore throat due to Klebs-Loeffler's bacillus infection Cancrum oris is not very infrequent and is a very serious complication too (Sen Gupta, 1947*b*)

Pneumonia is a frequent complication of kala-azar The condition recurs inspite of treatment until specific treatments of kala-azar are instituted (Dhar, 1938-39).

The other complications are (i) pneumococcal meningitis following pneumonia, (ii) otitis media (*Sen Gupta et al*, 1948) and (iii) purpura Gupta and Chatterjee (1949) reported a case of kala-azar developing purpura.

Pathology :

The pathological changes taking place in the bone marrow during the acute, late subacute and late chronic stages of the disease were described in detail by Chatterjee (1946). The most important features of such changes were gradual absorption of marrow and its replacement by fat cells. The constant presence of leishmania parasites in the clasmocytis was also a very important finding.

Biochemistry :

Hepatic functions in kala-azar cases were tested by six different tests such as,

hippuric acid synthesis and Takata-Ara tests, prothrombin time, serum-colloidal-gold reaction and estimation of serum proteins. The results showed liver hypofunction in kala-azar especially towards the later phase of the disease (Chakravarty *et al*, 1949; Chakravarty, 1950).

The excretion of urinary chlorides increases and the concentration of the plasma chloride falls in kala-azar. Blood sugar, urea and non-protein nitrogens generally do not show marked fluctuations from the normal, but blood cholesterol, on the other hand, becomes subnormal in a large percentage of cases (Chakravarty *et al.*,1949).

Diagnosis :

The diagnosis of kala-azar may be made in a proportion of cases by microscopic examinations of peripheral blood films (Napier, 1939*b*), sternal and spleen puncture materials, by serological tests as well as by cultures. Spleen puncture gives a greater percentage of positive results than sternal puncture although the former is more risky than the latter (Napier, 1939*a*; Reddy and Subramanian, 1939). Lowe (1946) prefers sternal puncture and reserves spleen puncture for the diagnosis of obscure cases.

The serological tests include : (i) Chopra's antimony and Napier's aldehyde tests and (ii) complement fixation tests. Chopra's test becomes positive earlier than the aldehyde test When the spleen is markedly enlarged the aldehyde test is more reliable than the antimony test (Napier, 1939*b*). Aldehyde test, however, has some limitations and is liable to be positive in some other diseases also (Laha, 1940, Raman, 1944). Raghavan (1949) and Raghavan and Satyaprakash (1949) introduced some modifications in aldehyde and Chopra's tests in order to make these suitable for mass examination of kala-azar sera in the field.

Two types of antigens are available for complement-fixation tests of which one is non-specific (W K K antigen) and the other, specific. When W.K.K. antigen is used, the test becomes positive within one month when aldehyde and Chopra's tests are generally negative. The diagnostic value of the test is high. It is specified for kala-azar and is negative in all diseases except obvious pulmonary T.B. and severe leprosy (Greval *et al.*, 1939; Sen Gupta, 1943*a* and 1944*b*, *c*). The W.K.K. antigen is prepared from tubercle bacilli. Dharmendra *et al.* (1946), however, employed the more easily cultivated acid-fast bacilli of Kedrowsky and Lloras. This modified W.K.K. antigen has proved to be as specific and sensitive as the original one (Sen Gupta, 1944*c* and 1945*a*).

The specific antigen is prepared by extracting cultures of *L. donovani* grown on Ray's solid medium with distilled water containing 0 5 per cent phenol. This antigen is specific and has given highly satisfactory results (Niyogi and Ray, 1942; Ghosh *et al*, 1945 and 1949).

For the diagnosis of kala-azar, blood and also spleen and sternal puncture materials may be cultured. An well-equipped laboratory is essential for this purpose (Napier, 1939*b*). Patkar (1949) claimed that if Row's medium be selected and the tubes after inoculation are kept at 24°C, the growth of the parasites would be evident within a fortnight In this connection it may be stated that the leishmania parasites are able to grow in symbiosis with *Myc. tuberculosis* (Row, 1950).

When laboratory facilities are absent diagnosis may be made on clinical grounds and confirmed by response to antimony treatment (Saxena, 1939). Xonodiagnosis (Shortt, 1945) is very seldom employed.

Treatment :

Antimony compounds In the treatment of non-resistant and uncomplicated cases, antimony compounds are the drugs of choice Several new antimony compounds have recently been introduced into the market and these are (i) Neostibin, a derivative of *p*-amino-phenyl-stibaminic acid (Brahmachari, 1941), (ii) sodium antimony-V-gluconate (stibatin of Glaxo Laboratories), and (iii) methyl glucamin antimoniate (a pentavalent antimony compound). Sodium antimony-V-gluconate is remarkably non-toxic and has many advantages over neostibosan or urea stibamine. It has been found to be the most useful drug in the treatment of kala-azar (Patel, 1944; Bruke and Chakravarty, 1944; Chakravarty, 1945; Sen Gupta and Chakravarty, 1945*b*; Ghosh Dastidar, 1945; Chowdhuri, 1946). Regarding methyl glucamine antimoniate, it may be said that its toxicity is low and thus it may be safely used (Sen Gupta, 1950*a*).

Diamidine compounds Stilbamidine or diamidino-diphenyl-ethylene is useful, particularly, in the treatment of antimony resistant cases. Although stilbamidine has the most powerful therapeutic actions, it is not suitable for treatment in outpatients' clinics due to the alarming reactions it produces immediately after the injection. It is reserved for the treatment of resistant cases only (Napier and Sen, 1940; Napier *et al*, 1942, Napier and Sen Gupta, 1943).

Stilbamidine may be replaced by hydroxystilbamidine although the latter is not so powerful as the former. Hydroxystilbamidine does not give rise to the characteristic immediate reactions of stilbamidine nor to the neuropathic sequels (Sen Gupta, 1949*a* and 1950*b*).

Pentamidine (diamidine-diphenoxy-pentane) is the second member of the diamidino group of compounds. It has some curative action against ordinary as well as resistant cases. It is inferior to diamidino-stilbene and also to the best of the pentavalent antimonials. Immediate reactions follow intravenous injections but are much less severe than those produced by diamidino-stilbene (Sen Gupta, 1944*a*; Napier and Sen Gupta, 1943). Pentamidine isethionate is equally effective. Im-

mediate reactions after its injection are mild (Ghosal and Sinha, 1948; Hazarika, 1949).

Phenamidine or diamidino-diphenyl-ether is the third member of the group. It is inferior to the most effective antimony compounds and stilbamidine. Being non-toxic it can be safely used where antimonials are contra-indicated (Sen Gupta, 1944*d* and 1945*b*).

For the resistant cases stilbamidine is the drug of choice. If it fails, splenectomy is advised (Sen Gupta, 1949*b*) although this is not always a guarantee for cure (Das and Sen Gupta, 1950).

In kala-azar a balanced diet with full complement of vitamins is prescribed (Sen Gupta, 1949*b*).

Toxic reactions follow after intravenous injections of urea-stilbamine and stilbamidine. The reactions are rare after urea-stibamine, but develop constantly after stilbamidine. The toxic reactions of urea-stibamine are : rash formation, unconsciousness and sometimes haemorrhages (Das Gupta, 1939). Stilbamidine gives rise to the development of vasomotor type of disturbances, such as, fall of blood pressure, flushing of the face, vomitting dyspnoea, sweating and collapse. The symptoms develop immediately (Napier and Sen 1940; Napier *et al*, 1942). The neuropathy is rather a sequelae and appears 3 to 4 months after the injection (Napier and Sen Gupta 1942, Sen Gupta 1943*b*).

It has been observed that when dogs are injected intravenously with a solution of stilbamidine previously autoclaved at 5 lb pressure for 20 minutes, their central nervous systems are damaged. This observation has some value as it may shed light on the causation of neuropathic manifestations in man According to Sen Gupta (1947*a*) the central nervous system lesions in dogs are due to some changes in the drug caused by high temperature of the autoclave.

Treatment of complications :

Tuberculosis : When tuberculosis complicates kala-azar diamidino-stilbene is recommended, as it does not aggravate tuberculous process. Along with diamidino-stilbene, streptomycin has also been used as the anti-tuberculous agent (Sen Gupta, 1944*b* and 1949*a*).

Cancrum oris · Penicillin works wonder when it is combined with specific drugs of kala-azar. The antibiotic is injected I M. and also applied topically. As supporting measures, blood transfusions and intravenous glucose injections are essential (Sen Gupta and Chakravarty, 1945*a*, Sen Gupta, 1949*b*).

Granulopoenia . In severe granulopoenia, Banerjee (1950) advocates the following injections : (i) organic antimony compounds such as, Neostibin; (ii) pen-

tose nucleotide; and (iii) blood (as transfusion). If the sore throat is due to Kleb's-Loeffler's bacillus infection anti-diphtheretic serum is also given.

Pneumonia: In treating pneumonia anti-pneumococcal drugs and antimony compounds should be given together (Dhar, 1938-39).

Neuropathy: The only treatment for this troublesome condition is the injection of 1 : 1,00,000 solution of cobra venom in gradually increasing doses (Sen Gupta, 1943*b*).

Toxicity:

Bose *et al* (1946) showed that when the ratio between the quantities of antimonious acid and antimony present in a sample of urea-stibamine exceeded 1 : 26, the preparation became toxic The toxicity of other compounds such as the salts of diethylamine and isopropyl amine were due to some impurities.

White rat and white mice are generally employed for bioassay of antimony compounds. Pigeons may also be used for this purpose (Dutta *et al*, 1946). The LD_{50} of urea-stibamine in white mice is 215 mg kg. (Bose *et al*, 1945).

Pharmacology:

The excretion of antimony from kala-azar patients is considerably delayed as a result of renal hypofunction. This was demonstrated by Chakravarty and Sen Gupta (1950).

Immunity:

Rabbits immunized by intravenous injections of live cultures of *L donovani* develop in their sera complement-fixing and agglutinating antibodies in high titres. No separate 'H' and 'O' agglutinins have been detected (Ghosh and Ghosh, 1947).

Control of kala-azar:

Attmepts to destroy the larvae of sandflies are likely to be unsuccessful as the former are found in totally different environments spread over wide areas In villages where kala-azar and malaria co-exist, measures against the adults are likely to give the best results as the adults of both sandflies and mosquitoes take shelter in houses together (Smith and Ahmed, 1941). The adults may be dealt with by: (a) spraying of houses and the surroundings with insecticides and (b) cultivating cleared areas (Bruke, 1943).

Kala-azar may also be controlled by isolation and treatment of the sick. For

test is essential. A careful watch on contacts for the first signs of the disease is also essential (Bruke, 1943; Rogers, 1939; Prasad, 1949; Shortt, 1945).

Post-Kala-Azar Dermal Leishmaniasis

Clinical .

The types of skin lesions observed in post-kala-azar dermal leishmaniasis are the following. (a) nodules on the chin, trunk, external genitalia, tongue and cornea; (b) ulcers on the different parts of the body; (c) ichthyotic condition of of the skin, (d) dark coloured depressed scars around the mouth, on the cheeks, eyelids and at the canthi of both eyes; (e) hypo- or dipigmented patches on the face, trunk, limbs and on the mucous membrane of the palate, and (f) erythema of the face (Ghosh Dastidar, 1939, Napier and Kirwan, 1941; Brahmachari and Basu, 1942; Brahmachari, 1942 and 1943*a*, *b*; Sen Gupta *et al.*, (1950).

In Assam, Pande (1941) came across a case of kala-azar in a bullock showing nodules and ulcers on the body.

Pathology :

The skin lesion starts as an erythema It then undergoes a series of changes until a red nodule is formed (Brahmachari, 1943*b*). Sen Gupta *et al.* (1950) have described in detail the histological changes that take place in a nodule.

The parasites are rarely found in erythematus and dipigmented patches, but are always abundant in the papules and nodules. They are contained within dermal melanopheres, the large extravascular cells, monocytes, histiocytes etc. (Brahmachari, 1942).

Treatment ·

Some cases respond to antimony compounds, while others do not (Brahmachari, 1943*a*, *b*; Sen Gupta and Chakravarty, 1945*b*). Ulcers, icthyotic skin lesions and leishmanial keratitis improve after injections of organic antimony compounds (Brahmachari and Basu, 1942; Napier and Kirwan, 1941). In keratitis, potassium iodide is also given.

It is not possible as yet to estimate the value of diamidino-stilbene in the treatment of post-kala-azar dermal leishmaniasis although Napier and Sen (1940) have observed distinct clinical improvements in a case after 20 injections of these drugs.

Oriental Sore

Epidemiology :

- In Delhi Oriental sore is sporadic. In the year 1939 there was an epidemic when along with human beings dogs were also infected (Shah, 1941). Cases have

been recorded in Kasubegu near Ferozepore and in Pattam and Jalan in Hyderabad (Chopra, 1943; Daver and Ahmed, 1943).

Transmission :

Although it has now been established that sandfly, *P. papatasii* transmits Oriental sore, Elkerton (1944) suggests that *Stomoxys calcitrans* may play some part.

Clinical :

The incubation period is about six months (Elkerton, 1944). The sore may vary in number. In a labourer, Shah (1941) counted as many as 230 sores in addition to scars of many healed up ulcers

Treatment :

In addition to local, treatment by injections of antimony compounds may also be given (Shah, 1941). Patients suffering from Oriental sore, react very badly to diamidino-stilbene (Napier and Sen, 1940). Local treatment consists of scraping and dressing, intradermal infiltration and X-ray application. After scraping the sore under short general anaesthesia, it may be dressed with tannic acid powder, magnesium suphate paste, or 4 per cent tarter emetic, weak carbolic and atebrin ointments or ointment containing alum (Shah, 1941, Elkerton, 1944).

For infiltrating the sores solutions of quinacrine (atebrin) in distilled water or of berberin sulphate (Orisol) may be used, the latter is more effective than the former (Sachdeva, 1943; Elkerton, 1944; Chopra, 1943). It has been observed that a 0.5 per cent solution of umbellatine, an alkaloid isolated from *Berberis umbellata* and *B. insignis* is therapeutically as active as a 2 per cent solution of berberin sulphate (Gupta and Kahali, 1944).

X-ray cures Oriental sore (Panja, 1946).

Diagnosis .

This may be made by : discovery of leishmania parasites from the sores, and by positive skin reactions. For skin test 'Leishmin.' presumably an antigen prepared from cultures of *L. tropica* is used. The test is positive in persons suffering from Oriental sore and also in those who have been exposed to the risk of infection (Shah, 1941).

Pharmacology:

Gupta and Kahali (1944) have shown that while a 1 : 50,000 solution umbellatine inhibits the growth of *L. tropica* in liquid medium, the drug at five time the above strength has no effect of *L.donovani.*

Culture :

When the cultures become old the flagellates of *L. tropica* transform into leishmania forms. These have been styled as "O-bodies". The transformation is due to conversion of oxyhaemoglobin in the culture medium into methaemoglobin (Row and Kulkarni, 1942).

From bacterially contaminated sores the parasites may be isolated in pure form if penicillin be added to the culture medium prior to inoculation of the material from the sore (Mukherjee, 1945).

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RELAPSING FEVERS

Tick-Borne Relapsing Fever

Epidemiology:

Tick-borne relapsing fever is prevalent in Kashmir in the Provinces of Jammu and Kashmir excluding Ladakh Valleys (Rao and Kalra, 1949). The tick-borne relapsing fever of Kashmir is milder than the louse-borne relapsing fever of India (Narain and Kalra, 1950). The incidence is highest during summer months (Rao and Kalra, 1949).

The disease is transmitted by *Ornithodorus crossi*. This tick readily feeds on laboratory animals such as, guinea-pig, white rat, white mouse and monkey (*Macaca radiata*) and infect them Goat, however, is resistant to infection (Rao and Kalra, 1949).

Clinical

The incubation period varies between 5 and 15 days The symptoms are local and general The local symptom starts as itching This is followed by the formation of a small papule with a tiny vesicle The vesicle soon ulcerates. Within a few days the papule enlarges and then becomes darkly pigmented and lichinified The general symptoms are, fever, muscular pain, headache, giddiness and prostration The liver and spleen are enlarged, the former being also tender (Kaul, 1949). The paroxysms last from 1 to 7 days and the relapses occur at intervals of 1 to 14 days, generally 2 to 4 days (Narain and Kalra, 1950)

Complications:

These are (i) iritis, and (ii) effusions in knee and elbow joints (Kaul, 1949). The other complications such as, haemorrhage, pneumonia, rash, nervous sequelae and secondary anaemia are absent in tick-borne relapsing fever of Kashmir (Narain and Kalra, 1950)

Diagnosis:

This is made by microscopic examination of thick blood films (Kaul, 1949), and inoculation of susceptible animals with patient's blood (Narain and Kalra, 1950). W.R. and Kahn tests are generally negative (Kaul, 1949).

Treatment:

Ninety-seven per cent of cases respond to arsenical compounds such as N A B Penicillin has no effect. The arsenic-resistant cases are resistant also to penicillin. Streptomycin, on the other hand, is highly active against relapsing fever spirochae-

tes including the arsenic-resistant strains (Narain and Kalra, 1950; Kaul, 1949).

Control:

Kalra, Jacob and Rao (1950) studied the effects of DDT, gamma BHC and Pyrithrin on *Ornithodorus crossi* in Kashmir In the laboratory these insecticides were used as dust (DDT and gammexane 929), as emulsions (DDT), solutions in kerosene (DDT and gammexane Lg 140) and in water suspensions (Gamma BHC). All were active against the ticks excepting two, viz., DDT emulsion and pyrithrin solution. When used in the fields DDT and gamma BHC gave satisfactory results, but reinfestations occurred as soon as the insecticidal properties were lost

Louse-Borne Relapsing Fever

Nine cases of louse-borne relapsing fever were detected among labour recruits in north-eastern Bengal (Chakrabarty, 1949).

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Rat-bite Fever

Epidemiology.

Rat-bite fever is not uncommon in Calcutta and Bombay In Calcutta the incidence is highest during hot weather from May to September (Chopra *et al.*, 1939). Rat-bite fever may also result from the bite of Indian squirrel (Sciurus sp.). A case has been reported by Das Gupta (1942).

Clinical:

Vakil (1943) has described two cases of rat-bite fever in whom besides the usual clinical manifestations of the disease, multiple gummatoid lesions developed within the muscle and the periosteum.

Diagnosis

Diagnosis is made by the presence of *Spirillum minus* in the exudate from the site of the bite (Chopra *et al.* 1939), inoculating patient's blood in susceptible

animals such as guinea-pig or white mouse (Das Gupta, 1942; Chopra *et al.*, 1939) and by response to N A.B. (Chopra *et al*, 1939, Das Gupta, 1942).

When blood examinations fail to detect the *Spirillum*, centrifuged deposit from 1 c c. of patient's blood may be injected intraperitoneally into a mouse (Row *et. al.*, 1941). After the inoculation the animal becomes infected within 12 to 26 days (Row *et al*, 1941; Das Gupta, 1942)

Treatment

Arsenic is specific in rat-bite fever. Among the arsenical compounds, namely, N A.B, Sulpharsenol, Solu-salvarsan and Sulpharasamine, N A B has been found to be the best There are some cases resistant to arsenic (Chopra *et al*, 1939)

Animal Experiments ·

The laboratory animals susceptible to *S minus* are, guinea-pig, white rat, white mouse and kitten. The incubation period, length of initial attack, number of relapses and longevity of these animals vary from one another It is not possible to produce infection by feeding (Basu and Sen, 1945)

Insect Vector.

Culex fatigans mosquitoes and rat-fleas do not play any part in the propagation of this infection (Basu and Sen, 1945)

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LEPTOSPIROSIS

Epidemiology

Leptospirosis occurs in Calcutta as well as in Bombay (Lahiri, 1941*b*). Lahiri (1943) recorded six cases of leptospirosis in Bombay City; the cases belonged to *L icterohaemorrhagica* group. They were found in different localities, had no occupational incidence, all were adult males (between 20-40 years of age) and lived in premises heavily infested with rats There is a case report indicating that leptospirosis occurs in Assam also (Das Gupta, 1941).

Clinical :

Although fever and jaundice are the two most common symptoms of leptospirosis, jaundice may sometimes be absent (Lahiri, 1945*a*) Mohanty (1945) reports a case suffering from haematemesis, melaena and jaundice. Although the serum from this patient failed to agglutinate classical strain on *L icterohaemorrhagica,* the centrifuged deposit from his urine contained spirochaetes closely resembling leptospirae.

Besides man, dog may also suffer from jaundice due to *L. icterohaemorrhagica* infection (Das Gupta and Sen, 1945)

Diagnosis :

This may be made by ·

(1) *Agglutination test* · Serum of persons suffering from leptospirosis agglutinate *L. icterohaemorrhagica* in high dilutions (Lahiri, 1941*b* and 1945*b* ; Das Gupta, 1941). Such sera may also agglutinate *L. canicola* and Andaman strains in low dilutions (Das Gupta, 1941). Das Gupta (1942*c*) reports of a strain of *L. Canicola* which he had maintained by subcultures in Vervoot's medium, agglutinated in low dilution in presence of serum from a case of leptospirosis as well as from normal human beings and many animals

(2) *Animal inoculation* : Guinea-pig is the animal of choice. White mouse (Haffkine Institute in-bred, Javanese and Swiss mice) is resistant to infection by the Indian strains of leptospira and are thus not suitable for diagnostic test of this disease (Lahiri, 1945*b* ; Das Gupta, 1942*b*).

In order to detect the existence of *L icterohaemorrhagica* infection in rats in Bombay city, Lahiri (1941*a*) employed four methods, viz., (i) direct examination of the convoluted tubules of the kidney with dark ground illumination, (ii) inoculation of guinea-pig with emulsion of the kidney from the suspected rat, (iii) culture of renal cortex and (iv) serological test of rat's sera. Combination of methods (i) and (ii) gave the best result.

Isolation ·

When *L icterohaemorrhagica* has been contaminated by bacteria such as *S. typhimurium*, the former may be isolated free from the bacteria by using bacteriophage potent against the contaminating bacteria. When tissues such as liver or spleen are present, purification may be done by filtration through kiesselguhr paper or by centrifugation (DeMonte and Gupta, 1941).

Transmission .

In animals the infection is transmitted from the mother to the foetus through

the placenta. Definite evidence on this point has been obtained by Das Gupta (1939*a*) in case of guinea-pigs

Immunity.

Active immunity develops after injections of leptospiral (*L icterohaemorrhagica*) vaccine. Vaccinated persons develop in their sera agglutinins and other antibodies which protect guinea-pigs from infection (Das Gupta, 1942*b*).

Immunity may also be passively transferred by injection of antisera raised against homologous as well as heterologous strains of *L icterohaemorrhagica* In case of homologous strain, the antisera offers protection from 23 to 41 days, while in case of serum from heterologous strain infection, the protection lasts for 10 days only (Das Gupta, 1939*b*, *c*). The young of actively immunized guinea-pig acquires passive immunity which persists for 2 months generally but not beyond 6 months (Das Gupta, 1939*b*, *c*)

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TRYPANOSOMAS

A *Culex fatigans* mosquito infected with *P. relictum* by feeding on a wild house sparrow showed trypanosomes in addition to sporozoites of malarial parasites, in the salivary glands. Two morphologically different types of trypanosomes were seen As regards their origin it has been suggested that the trypanosomes might have been derived from the sparrow on which the mosquito was fed although the sparrow itself did not show trypanosomes in the blood (Singh *et al*, 1950).

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Yaws

Certain observations on yaws were made by Sinha (1939) in Chatra sub-division of Hazaribagh District, Chhotanagpur and by Sen Gupta (1946) in Bastar State. Rajan (1938) reports a case of early acquired syphilis where longstanding yaws had failed to confer any immunity against the subsequent syphilis. Iswariah and Nair (1938) reports an epidemic of an infection with an organism morphologically indistinguishable from *Sp. pallida* which affected a number of women and children in a Madras village. They discussed the question of this disease being syphilis or yaws or a separate condition such as Bejel as described by Hudson.

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INTESTINAL PROTOZOAL DISEASES

Amoebiasis

Epidemiology:

The incidence of amoebiasis is high in India. This is evident from the records of hospitals and out patients' clinics (Mayer, 1940, Chaudhuri and Rai Chaudhuri, 1946, Patel, 1945). The infection does not spread uniformly everywhere in India The different climatic and other conditions prevailing in the different parts of India may have some influence on the epidemiology of the disease. This has been demonstrated by Blumenthal *et al*, (1947) During the last war, while the incidence among the American troops stationed near Calcutta was 20 per cent among those staying in New Delhi it was 5 per cent only

Amoebic hepatitis is by no means rare in India (Banker, 1947). Reddy and Thangavelu (1948) have shown that 30 per cent of all hepatitis cases treated in the Government Hospital, Madras were amoebic in origin.

Amoebiasis does not generally show seasonal incidences (Reddy and Thangavelu, 1948)

Excessive rainfall, insanitary surroundings and increased fly breeding encourage the spread of amoebic infection (Lawrence and Bennett, 1945) The organisms are carried by faecally contaminated fingers of patients and carriers engaged in household work (Malhotra, 1949) and also by contaminated foods, dust and flies

The carrier rate in Bombay is about 43 3 per cent and in Calcutta 49 per cent (Patel, 1945, Chaudhuri and Rai Chaudhuri, 1946).

Aetiology:

Amoebiasis (intestinal and hepatic) is most prevalent in the ages between 20 and 50 years (Chaudhuri and Rai Chaudhuri, 1946, Banker, 1947, Reddy and Thangavelu, 1948) The extremes of ages are 2 years and 60 years (Chaudhuri and Rai Chaudhuri, 1946, Banker, 1947) In children the incidence, however, is low (Reddy and Thangavelu, 1948). Patel (1945) reports of a case of amoebic infection in a child of 6 months

Males suffer more than the females (Banker, 1947, Reddy and Thangavelu, 1948). It is more prevalent among the poor labour class people (Banker, 1947).

Although amoebiasis is very common among Indians living in insanitary conditions, Anglo-Indians and Europeans are also not spared (Chaudhuri and Rai Chaudhuri, 1946) Hindus are more prone to liver infection than the Muslims (Reddy and Thangavelu, 1948).

Pathology:

The lesion starts as an ulcer at caecum from where it spreads into the whole of the large intestine Appendix rarely, though rectum is more frequently involved (Reddy and Thangavelu, 1948). From the intestine metastatic spread may take place into liver, lung, brain and spleen. Besides ulceration, amoebic infection may also give rise to the formation of granulomatus tissues (amoeboma) (Reddy and Rangam, 1945). Amoebic ulcers may co-exist with ulcers of typhoid (Reddy and Thangavelu, 1948) Chaudhuri and Chakravarti (1946) recorded a case of carcinoma of the colon infected with *E. histolytica.*

Regarding amoebic ulcers Banker (1947) observed that the abscess contents in 78 per cent of cases were typically chocolate coloured, but in the remainder it was brownish yellow. Trophic forms of *E histolytica* may be found in the abscess content.

Liver function ·

The liver function tests indicate that the functional derangements of the liver are more among those suffering from amoebic dysentery than those from amoebic hepatitis (Heilig and Visveswar, 1944).

Blood changes

Apart from leucocytosis which is a common occurrence in liver abscess (Banker, 1947) there takes place an alteration of plasma proteins both in intestinal and hepatic amoebiasis This has been demonstrated by Chakravarti (1950).

Diagnosis ·

Diagnosis of amoebic infection is made by microscopic examinations of stool, rectal swab and discharge and scrapings from other places. Stool does not always give reliable information When the number of cysts is very small the copper sulphate floatation technique may be employed In order to float the cysts the stool emulsion is mixed with a copper sulphate solution of specific gravity 1.035.

The rectal swab is valuable especially in children suffering from diarrhoea. When rectal swabs are examined the trophozoites may be found even when these are absent from the stool (Coelho, 1949). In amoebic vaginitis, the discharge from the vagina shows the amoebae (Sen, 1949).

For the diagnosis of extra-colonic amoebic infections there are no reliable tests. However, in these cases complement-fixation tests may be tried In performing complement-fixation tests carbol saline extracts of pathogenic amoebae from cultures are used as the antigen (Ghosh *et al.*, 1948).

X-ray is useful in the diagnosis of liver abscess. Immobility and raising of the right dome of diaphragm are suggestive of liver affection The presence of a dense, central and circular area will confirm the diagnosis (Banker, 1947). In chronic obscure liver conditions, pneumo-hepatography may be performed (Clark and Dutta, 1945).

In absence of positive laboratory findings diagnosis is made clinically and confirmed by response to emetine (Mohapatra, 1947).

Clinical :

When the alimentary tract is affected, amoebiasis gives rise to (i) dyspeptic (Banerjee, 1939, Patel, 1945, Chaudhuri and Rai Chaudhuri, 1946), (ii) dysenteric (Mayer, 1940); and (iii) appendicular symptoms (Banerjee, 1939, Ghosh, 1948). According to Chaudhuri and Rai Chaudhuri (1946) about 63 8 per cent of cases suffering from intestinal amoebiasis show dysenteric manifestations while the rest dyspepsia

Sometimes granulomatous tissues develop inside the large intestine having amoebic ulcers and give rise to symptoms of intestinal obstruction Several cases of amoebic granuloma have been reported (Chaudhuri and Rai Chaudhuri, 1946; Reddy and Rangam, 1946) Besides the large intestine the amoeboma may also develop on the skin (Ghosh and Mukerji, 1950).

The symptoms of amoebic hepatitis (or abscess) are, pain and tenderness in the right hypochondrium and enlargement of liver (Banerjee, 1939) Forty per cent of liver abscess cases give a previous history of dysentery and in 3 per cent, the dysenteric symptoms coincide with liver abscess The symptoms such as shoulder pain, ocular icterus and persistent hiccough are not constant (Banker, 1947)

Amoebic abscess in the lung may be single or multiple (Chatterjee and Sen Gupta, 1949). Several cases of pulmonary amoebiasis have been recorded (Chaudhuri and Rai Chaudhuri, 1946) Mukerjee (1949) has classified pulmonary amoebiasis into the following types (a) symptomatic, (b) secondary, and (c) primary Primary lung abscess has been divided into (i) amoebic pneumonitis, and (ii) pulmonary amoebic necrosis.

Amoebic infections of brain, heart and spleen are rare. Koshy (1950) reports of a case of cerebral abscess, the abscess developing on the lower part of the left frontal lobe Pericarditis due to *E. histolytica* infection has been reported by Laha (1946). Amoebic abscess may develop in spleen (Singh, 1949).

Besides the above, amoebic infections may be the cause of ulcers on the skin surrounding the anal orifice (McConaghey, 1945; Rajam and Rangiah, 1939) and inflammation and ulcers on the mucous membrane of vagina, cervix, etc. (Balsubrahmanyan and Cheriyan, 1949; Sen, 1949).

Complications :

These are the following : (a) Intestinal obstruction and intussusception (Reddy and Rangam, 1946), (b) rupture of liver abscess into (i) right pleural cavity or lung, (ii) peritoneal cavity, (iii) both pleural and peritoneal cavities (Banker, 1947), (iv) pericardium (Singh, 1946; Reddy and Thangavelu, 1948) or (v) rectus sheath (Reddy and Thangavelu, 1948), (c) secondary infection of liver abscess (hematogenous or following aspiration) (Banker, 1947; Kark, 1945), (d) secondary lobar or broncho-pneumonia or pulmonary tuberculosis (Banker, 1947), and (e) deep jaundice (Reddy and Thangavelu, 1948; Reddy and Rangam, 1945) Bacillary dysentery may complicate amoebic dysentery (Lawrence and Bennett, 1945).

Treatment .

In amoebiasis emetine is specific It is indicated in acute dysentery, acute exacerbation of chronic amoebiasis, acute hepatitis and liver abscess (Chopra, and Chopra, 1942). It is also useful in amoebic lung abscess (Chaudhuri and Rai Chaudhuri, 1946, Mukerjee, 1949), vaginitis (Sen, 1949, Balsubrahmanyan and Cheriyan, 1949), and in amoebic granuloma (McConaghey, 1945). Rajam and Rangiah (1939) advise that when emetine injections are completed these should be followed by a course of E B I In the treatment of chronic amoebiasis E B I. is recommended (Chopra and Chopra, 1942).

The arsenical preparations, such as, carbarsone, stovarsol and 4,876 are valuable in acute as well as chronic intestinal amoebiasis. Among these three, carbarsone is therapeutically most active (Chopra and Chopra, 1942; Chaudhuri and Rai Chaudhuri, 1946). *p*-carbamido-phenyl-arsenious acid (C P.A) is a trivalent arsenical compound. C P.A. has been claimed to be more effective than carbarsone. However its greater toxicity limits its usefulness as an amoebicidal drug (Bose *et al* , 1950)

The quinoline derivatives such as, enterovioform is comparatively non-toxic amoebicidal drugs. This may be prescribed in acute, subacute and also in chronic intestinal amoebiasis (Mayer, 1940; Chopra and Chopra, 1942) Enterovioform may be given concurrently with emetine (Mayer, 1940). Although enterovioform is non-toxic long continued use may give rise to toxic symptoms. Pal *et al* (1944) tested Indian-made quinoline compounds and observed that these compounds agreed closely with enterovioform.

Amesanil maltex is a quinoxyl compound. It may be tried with satisfaction in chronic amoebiasis (Jacoby, 1946)

The other drugs for the treatment of intestinal amoebiasis are · *Holarrhena antidysenterica* and (Kurchibark) Yatren or Chiniofon. Clinical experience suggests

that *H. antidysenterica* (Kurchi) is a good remedy for both acute and chronic amoebic dysentery (Chopra and Chopra, 1942, Chaudhuri and Rai Chaudhuri, 1946). The alkaloids of this plant are poisonous and, as such indiscriminate use of *H antidysenterica* may produce fatal consequences. Dutta *et al*, (1950) noticed that the alkaloid contents of the different parts of the plant varied seasonally Yatren given by mouth is also a good amoebicide (Chaudhuri and Rai Chaudhuri, 1946)

Retention enemas:

Gross (1947) suggests that as amoebiasis is a local infection of the large intestine, E B.I may be applied topically as small retention enemas Retention enemas with Yatren have been advocated by Chaudhuri and Rai Chaudhuri (1946).

Diet

Diet in chronic amoebiasis should be so chosen that it should persistently keep the reaction of the stool alkaline (Ghose, 1939) Alkaline reaction of stool, it is said, inhibits the growth of the amoebae.

Surgical treatment.

The treatment of intestinal obstruction is medical if the obstruction is partial but if it is complete, surgical interference is essential (Reddy and Rangam, 1946). The surgical treatment of amoebic liver abscess lies in the evacuation of abscess cavity by aspiration This is to be followed by injections of emetine When aspiration fails the abscess requires to be opened and drained In case of secondary bacterial infection of the abscess sulpha drugs may be given by mouth. It is better, however, to inject penicillin directly into the abscess cavity after the aspiration (Kark, 1945).

Immunity

Chronic amoebiasis inhibits the formation of antibodies against bacterial infections (Ghosh, 1940).

Control measures.

These are (i) improvement of sanitation, (ii) fly control by spraying, trapping, screening of buildings, etc, and (iii) prophylactic administration of carbarsone to all suspected to be harbouring amoebae in the intestine in doses of gr. ½ daily for 10 days (Lawrence and Bennett, 1945). (a) disposal of faeces, (b) protection of water supplies from faecal contamination, (c) carriers not to handle food and drinks.

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FREE-LIVING AMOEBA

Singh (1950) studied nuclear division in free-living amoebae. After culturing the parasites on a thin film of an agar medium the film was fixed, agar removed by gentle shaking and then stained and examined

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GIARDIASIS

Epidemiology.

Giardiasis is steadily increasing. This has been based on the records of Khulna District Board Laboratory, India It is said that giardiasis exacerbate once every four years (Das Gupta *et al*, 1944)

Children are more susceptible than the adults (Chopra *et al*, 1939, Mohapatra, 1948) Coelho (1949) reports of giardiasis in infants of ages as low as 14 days and 1 month respectively Both sexes are equally susceptible

Pathogenesis

It has now been confirmed that *Giardia intestinalis* causes intestinal disturbances (Chopra *et al*, 1939). The normal habitat of the trophic forms is duodenum Apart from duodenum the parasites may also invade the gall-bladder and give rise to cholecystitis (Bhattacharya, 1943). Treu (1944) claims that the parasites may infect the large in addition to the small intestine.

Diagnosis

Diagnosis is made by examination of stool and rectal swabs. Cysts are generally found in the stool More information is supplied by rectal swab than stool (Coelho, 1949).

Duodenal incubation detects the presence of trophozoites in the duodenal content

and also in the 'B' fraction of bile In the 'D' fraction the number of flagellates are reduced (Bhattacharya, 1943).

Clinical.

There may be no symptom in giardiasis; but when present they simulate those of (i) dyspepsia (Treu, 1944, Bose *et al*, 1944, Mohapatra, 1948), (ii) diarrhoea (Treu, 1944), (iii) dysentery (Treu, 1944; Das Gupta *et al*., 1944), (iv) cholera: R B C s and pus cells are, however, present in the stool (Das Gupta *et al*., 1944), (v) peptic ulcer Fractional test meal shows low acid curve in such cases (Bhattacharya, 1943), (vi) spure (Treu, 1944, Chaudhuri, 1943) and (vii) bronchial asthma (Sirker, 1949).

Treatment.

Atebrin (Mepacrine) is specific in the treatment of giardiasis (Bhattacharya, 1943; Chopra *et al*, 1939, Mohapatra, 1948, Sirker, 1949). It may be administered in the same doses as are usually given for the treatment of malaria (Chopra *et al*., 1939) The butyl acridine compound with the formula 2-chloro-7-methoxy-5 (8-diethylaminobutyl) amino-acridine, is also effective in giardiasis (Bose *et al*., 1944). The other drugs that may be tried in giardia infection are stovarsol, N.A.B. or emetine (Bhattacharya, 1943; Gour, 1949).

For the relief of dyspepsia, acid mixture and for anaemia liver extract may be given. Although in spure-like conditions acid mixtures and liver extract are useful and these have no effect on steatorrhoea for which Mepacrine is essential (Chaudhuri, 1943).

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BALANTIDIASIS

A fatal case of balantidial dysentery has been recorded by Miller and Peck (1948) in Assam. The symptoms in this case were pyrexia, abdominal pain,

diarrhoea and dehydration. No pathogenic organisms were found in the stool. *Balantidium coli*, however, was isolated from an ulcer in the pelvic colon.

In the treatment of balantidial dysentery Hydrarg biniodide may be tried (Pramanik, 1947 and 1948).

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Other Protozoal Infections

Besides *Entamoeba histolytica* and *Giardia intestinalis* stools of persons living in India may show other intestinal protozoa such as *E coli*, *trichomonas*, *enteromonas*, etc Double, triple and quadruple infections may be seen (Chaudhuri and Chaudhuri, 1946, Jhatakia and Mankad, 1946, Patel, 1945)

Chopra *et al*, (1939) have shown that atebrin (Mepacrine hydrochloride) has no influence on *chilomastix*, *triochomonas*, *enteromonas*, *E coli* or *E histolytica*.

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II. REVIEW OF HELMINTHIASIS

HELMINTHIASIS

Hookworm :

Maplestone and Mukerji (1940) gave an account of the hookworm infection in India and described in brief the symptomatology, the lines of treatment and the prophylaxis. The highest incidence of the disease was found in Sylhet and Cachar in Assam, the tea gardens of the Assam Valley, the Dooars and the Darjeeling District, Travancore and Kanara (South India) A moderate infection is found in central Behar, eastern part of the U P, some parts of the Surma Valley and the foot hills of the Himalayas In other parts, only a mild incidence was found although in some places, 80 per cent of the people may show the infection. *Ancylostoma duodenale* is the commoner species in the Punjab and the U P, while *Necator americanus* appears to be the prevailing species in the other areas. For treatment, they recommend tetrachlorethylene, 4 c c together with a saline purgative in empty stomach Hare (1940) came to the conclusion that installation of bore-hole latrine in coolie lines in Assam would ultimately reduce the hookworm infection by preventing re-infection Maplestone and Mukerji (1940) compared the anthelminthic actions of various anthelminthics against hookworms and showed that tetrachlorethylene was best because of the greater efficiency, lower toxicity, lower cost etc Napier, Das Gupta and Majumdar (1941) discussed the aetiology and the treatment of hookworm anaemia and concluded that this condition was mainly due to mechanical blood loss though additional factors may be working to bring about intestinal dysfunction resulting in failure to absorb iron or other necessary ingredients, and that treatment of either the anaemia or the infection by itself will not be enough to yield satisfactory results Wilkins (1942) evolved a glycerine-salt technique for enumeration of the hookworm eggs in stools by use of simple apparatus and claimed that the method was accurate enough for clinical purposes. Heilig (1942) discussed the pathological conditions of the heart in hookworm anaemia and concluded that the anaemic condition caused the changes in the heart, as improvement of the blood condition was followed by an improvement of the cardiac condition, although he admitted the possibility of a toxin secreted by the worms Heilig and Visweswar (1942) recorded their observations on the influence of the treatment of hookworm anaemia on gastric secretion in hookworm anaemia and discussed the correlation between the two conditions. Mukerji and Maplestone (1943*a*) found that 1 per cent solution of common salt could be used as a preservative and diluent of stools for quantitative estimation of the eggs present and claimed that this solution was better than any other that were used for field work, the eggs keeping very well up to ten days They recommended that 3 c.c. of faeces should be mixed with 87 c c. of the salt solution. Rogers and Dammin (1946*a*) recorded the incidence of hookworm infection in

the American troops in Assam and Burma. Mukerji and Bhaduri (1947) used the indigenous drugs *Butea* and *Embelia* for the treatment of ascariasis and claimed that these in doses of 60 to 180 gr were superior to santonin and as good as oil of chenopodium, and recommended them as these were very cheap. Mukerji and Matheu (1950) carried out a hookworm survey in Jharia Coal Field Settlement Area and found that the incidence of hookworm was higher in the underground workers than the others and that the average infection was not very heavy

Gnathostoma Spinigerum:

Maplestone and Bhaduri (1937) reported the fourth human case of gnathostoma infection in Bengal Mukerji and Bhaduri (1945) recorded a case of gnathostoma infection of the eye in a man in Bengal, the sixth case in India so far The worm was removed surgically from the anterior chamber of the eye and was found to be an immature form of Gnathostoma and appeared to be other than *G spinigerum* or *G hispidum*, probably the species recorded earlier by Maplestone Maplestone and Bhaduri (1940) made a survey of the intestinal helminth parasites of the pariah dogs of Calcutta and discussed their bearing on human parasitology They recorded some new forms in India for the first time and suggested that *Trichostrongylus* infection in man is more likely to be acquired from the association with dogs which were found by them to show the infection Maplestone (1941) pointed out that *Trichostrongylus* infection, sometimes, were found in man and the species involved is *T colubriformis*, and that there is often a confusion in the diagnosis of this innocent infection as the eggs resemble the hookworm eggs Maplestone and Bhaduri (1942) recorded occurrence of *Trichinella spiralis* in a cat in India — the first authentic record of the work in India.

Guineaworm ·

Trewn (1937) reported a case from which 526 guineaworms were removed in three years and suggested how the cases could be dealt with in ordinarily equipped hospitals Maplestone and Sundar Rao (1939) gave an account of the distribution of guineaworm infection in India and outlined the lines of prophylaxis and treatment. Moorthy (1942) reported a case who had repeatedly guineaworm infection and in whom he found on the leg a cyst containing calcified adult worms and embryos. Rao (1942) made a guineaworm survey in the Osmanabad District and suggested the steps to be adopted for prevention of the disease

Rishworth (1938) recorded an adult filarial worm of unknown species from the skin of a human subject. Maplestone (1938) examined this parasite and found it to be different from *Wuchereria bancrofti* and the absence of microfilaria from the blood of the patient did not make it possible to arrive at any further diagnosis On account of some morphological affinities with *Loa loa*, he suggested the name

Loa inquinenda for this form. Iyengar (1938) made a study of epidemiology of filariasis in Travancore. There he found cases of *Wuchereria malayi* and *W. bancrofti* from examination of blood films, the former was more common, rural and transmitted by *Mansonoides* mosquitoes which bred in the local tanks where *Pistia* plants were present Control of *malayi* infection was easy, according to him, by cleaning the tanks of the *Pistia* plants. Mitra (1938) recorded the presence of *microfilaria bancrofti* in the blood of a resident of Mikir hills, Assam, where *mf. malayi* was reported to be commonly found Iyengar (1939) described the staining techniques for studying the differences in morphology between *mf. bancrofti* and *mf malayi*. Chopra and Rao (1939) dealt with the chemotherapy of filarial infection and found that none of the drugs they tried, was found to be satisfactory except fuadin and soamin, which gave benefit in certain directions from clinical point of view. As regards the action on microfilariae soamin had none while fuadin had a purely temporary effect Rao and Maplestone (1940) described the adult of *microfilaria malayi* Brug, 1927 Rao (1940) made a survey of filarial infection in Ratanpur (C P) and found only *malayi* infection present, 16.23 per cent of the population showed embryos in the blood and *mansonoide* mosquitoes were the vectors Menon and Ramamurti (1941) made studies on the survival, growth and exsheathment of microfilarae *in vitro* in media and described the mechanism of exsheathment These authors held that the same mechanism occurred in the stomach of the mosquitoes. Napier, Das Gupta and Rao (1940) made a study of sternal puncture in filariasis and concluded that there was no evidence that the microfilariae took shelter in the bone-marrow or are destroyed there, nor was there any change in the bone-marrow due to this infection. Rao and Sukhatme (1941) made a study of seasonal variation in the incidence of filarial lymphangitis by statistical methods and found that the incidence was higher in the monsoon months Ramanamurthi, Menon, Ramamurti, Mahadevan and Rao (1941) recorded their views about the setiology, pathology and managements of the various forms of clinical filariasis in various stages and the complications thereof. Ray (1941*a*) discussed the surgical complications of filariasis and stated that operations should be undertaken only under certain conditions Iyengar (1938) reported a case of *bancrofti* infection in a rural area and found that *Anopheles philippinesis* was the vector. He found both malarial and filarial infections in the same mosquito in nature Menon and Ramamurti (1940) studied the behaviour of the infective filarial larvae with special reference to their mode of escape from the proboscis of the mosquito and penetration of the human skin. Rao (1942) made a filarial survey in Lakhipur and Bikanady tea gardens in Assam and found that *W. malayi* was more common than *W. bancrofti*. In 1945, Rao made another survey of filarial infection of Dhamda (C.P) and found *W malayi* to be present there. Harris and Summers (1945) described a concentrated method of demonstrating the microfilariae in the blood. The method consisted in collecting venous blood in a tube

containing heparin and then haemolysing it by saponin solution and collecting the embryos by centrifugalisation

Maplestone and Bhaduri (1937) pointed out that human cysticercosis (due to *T. solium*) was very rare in India while the same condition in the pigs, as found in the local abattoirs, is not so. The incidence of *T. solium* in man is also rare They conclude that there is a great deal to be known about the epidemiology of this parasite before the disease can be controlled Rao (1937) records a case who had epileptiform movements of the left forearm, the skiagram of the brain was negative while a cyst occurring in the deltoid of the other arm showed, on biopsy a typical cysticercus. Raman *et al*, (1950) reported seven cases of cysticercus of man from south India Sami (1938) drew attention to the high incidence of the hydatid disease in the south-west Punjab and about 29 per cent of the dogs harboured *Echinococcus granulosus* Roy (1938) reports a case of infection by *Bertiella studeri in a child from Bengal—the twelfth case reported from man* Chopra, Pasricha and Lal (1939) recorded a case of hydatid cyst of the lungs — a case which had been seen and diagnosed by them ten years ago, the Casoni reaction was still well marked. Chandra (1940) recorded a case of hydatid cyst in the neck in a boy of six Senekji (1941) isolated a polysaccharide, free from proteins, from the scolices of the hydatid cyst He claimed that this powder was stable and the solution gave more uniform results than hydatid fluid for the diagnosis of hydatid disease Greval, Chandra and Das (1941) prepared an antigen from the hydatid fluid for the complement fixation tests for diagnosis of the hydatid disease These authors recorded their observations of complement fixation tests with this antigen on hydatid diseases and associated conditions, by using this new antigen. Mukerji and Maplestone (1943*b*) discussed the treatment of taeniasis After one treatment, 28 cases out of 42 were cured by carbon tetrachloride, 30 out of 61 cases by tetrachlorethylene, 5 out of 19 cases by hexylresorcinol and from these results they recommended the use of carbon tetrachloride for the treatment of taeniasis Mukerji and Bhaduri (1944) pointed out that there was a suddenly increasing incidence of *Taenia solium* infection in Calcutta where it was rather rare before, and they think that this was due to the increase of the population of the city and the increased demand for pork

Anderson and Suri (1945) recorded a case of infection by *Schistosoma haematobium* in a boy who was said to have never been out of his native district in the Punjab and was only at Poona and next posted on military service at Rawalpindi They raise the question if this boy acquired the infection in India and that the snails of India must have acted as vectors to this parasite, a fact very important in view of the movements of troops between India and the countries where this infection occurs Mukerji, Bhaduri and Narain (1946) attempted to transmit the infection of *Schistosoma haematobium* and *S. mansoni* through the common Indian snails, but were not successful, and they were unable to explain the occurrence of

a few sporadic cases of *S. haematobium* infection which was reported from time to time

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In this note an attempt has been made to present a complete account of work done in India during the period between 1938 and 1950 on the insects and their allies that are responsible for transmission or causation of human diseases only. Control of disease-carrying insects by D D T, B H C and various other insecticides as well as by biological methods proved to be of paramount importance in the conservation of human health Recent attempts to eradicate malaria by killing Anopheles mosquitoes by D D T spray have been very promising The study of life-history, geographical distribution, bionomics, taxonomy, etc. of those insects are very useful in working out successful control measures A brief review of work on all these aspects is presented below Basu (1950) published a review of work done in India in all branches of applied Entomology

Insect Transmission of Diseases

Knowles and Basu (1943), Basu (1943) and Basu (1945) carried out extensive investigations to determine the different transmitting species of Indian Anophelines and the limits of atmospheric temperature and relative humidity between which transmission of malaria will take place Temperature appeared to be a more important factor in transmission than humidity Besides temperature and relative humidity other factors were also studied Basu (1939) studied the biology of the malaria parasite (*Plasmodium falciparum*) when experimentally transmitted through *Anopheles stephensi*. Chopra and Basu (1938, 1939) worked out the effect or various anti-malarial drugs upon the infectivity of patients to susceptible mosquitoes Basu (1947) recorded abnormal development of malarial oocysts in *Anopheles stephensi*. He (1947) also reported the frequency of distribution of gametocytes of Indian strains of malaria parasites as to its importance to mosquito transmission of the disease Basu and Sundar Rao (1939) worked out the various factors (temperature, humidity, density of microfilaria and others) involved in the mosquito transmission of filariasis in man. Sen and Minett (1945) succeeded in transmitting Anthrax through *Musca domestica*. Basu (1948) transmitted Anthrax through *Musca nebulo/Ctenocephalis felis* and *Hyalomma ægyptium* Mehta (1948) studied on typhus in the Simla hills and suspected its transmission through *Trombicula deliensis* Krishnan *et al* (1949) succeeded in effecting transmission of *Rickettsia orientalis* by the bite of larvae of *Trombicula deliensis* Swaminath, Shortt and Anderson (1942) reported transmission of Indian kala-azar to man by the bites of *Phlebotomus argentipes* Basu and Sen (1945) made a thorough investigation on rat-bite fever due to *Spirillem minus* and studied its probable arthropod transmission Rao and Kalra (1949) reported tick-borne relapsing fever in Kashmir and Kalra, Jacob and Rao (1950) tried to control the transmitting Ornithodorus ticks with the help of insecticides.

Insect Bionomics

Thomson (1940-42) studied on the behaviour of *Anopheles minimus*. Observations of Pal, Rajindar (1945) on the bionomics of *A culicifacies* are of interest. Knowles and Basu (1943) reported behaviour of *A stephensi*., Russell (1941, 1942) reported agglutinogenic properties of sporozoites and immunization of fowls against sporozoites

Insect Control

Puri (1947) in a series of papers published the action of insecticides on adult and larval forms of anophelines and the practical application of D D T for malaria control in rural and urban areas in India Covell *et al*, (1938) made an attempt to control malaria by the destruction of adult mosquitoes with insecticidal sprays. Covell (1939) recorded the result of his anti-malarial operations in Delhi Iyengar (1946) found out naturalistic control of the breeding of *Anopheles sundaicus* by means of *Eichhornia* cover. Basu (1944) reported malaria control at Izatnagar by anti-mosquito measures

Insect Survey

Barher and Rice (1938) conducted mosquito survey in Poona and its vicinity. Russell *et al*,(1938) reported mosquitoes of Pattukkottai Taluk. Rao (1941) conducted a rat flea Survey of Calcutta.

Insect Taxonomy

White *et al*, (1940) published the volume on Diptera VI Family Calliphoridae. Sharif's (1938-49) work on Fleas (Aphaniptera) is of interest. Sweet and Rao (1938) reported cross breeding of *A. Stephensi* type and *A. stephensi* Var *mysorensis* to find out the variation Menon (1938) described the egg of *A. varuna* and *A fluviatilis*. Sweet and Rao (1938) measured *A culicifacies* ova.

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III. REVIEW OF MICROBIOLOGY

CHOLERA

A wide difference of opinion was found to exist between different laboratories in regard to the serological character of certain vibrio strains isolated from amongst the Hedjaj pilgrims at El Tor in 1930. This incident casts doubt upon the existing methods of identification of true cholera vibrio, upon which our knowledge of the epidemiology, treatment, control and quarantine measures essentially depended. A fresh interest was thus created for carrying out intensive investigations on the problem in which the then Office International d'Hygiene Publique gave the directives to the various institutions interested in the work Since then a large amount of research work has been done on the subject which has materially advanced our knowledge of the character of the true cholera vibrio, chemotherapy of cholera and other associated problems. India has taken the major share in this work mainly through the munificence of the Indian Research Fund Association (now called Indian Council of Medical Research) The various lines of work carried out by Indian workers during the past 10 years (1938-1948) are described below under different heads.

SEROLOGICAL IDENTIFICATION

Taylor (1941), who co-ordinated the studies on serological identification of cholera vibrio in India, felt justified in taking up the decision that no series of cholera cases should be attributed to a vibrio of fixed serological type other than that of non-haemolytic O group I of Gardner and Venkataraman. Although the weight of evidence favours this conclusion in general, a number of facts have made its truth in this absolute form somewhat doubtful. For instance, in a group of 828 vibrio strains isolated from cases of cholera (Taylor, 1938), as much as 13.5 per cent were inagglutinable and serologically and biochemically diverse. In the outbreak of Assam (Pandit, 1938) inagglutinable vibrios were exclusively found. Similarly, Seal (1946) mentioned 9 cases with 4 deaths in a Bengal village (Ratanpur) in which NAG vibrios could be isolated In a series of 90 strains of the type of Rangoon Rough 1, twenty-six were from patients with cholera, thus indicating a causal relationship of this group of vibrio with cholera. Another source of doubt lay in the observation that sera prepared against strains from cases had agglutinated a higher percentage of case strains than carrier or water strains while the opposite were observed with sera against carrier and water strains.

Anderson (mentioned by Gardner and White, 1937) working in Assam had earlier found that in sporadic cases or in small epidemics in Assam, strains were isolated which agglutinated with H and O serum and not with O group I, but on examining a large number of colonies in such cases, a colony could be found

which agglutinated with O group I sera. It was, therefore, necessary to assume, according to this hypothesis, that this one was the cause of cholera while the vibrios isolated in large number at the same time were to be considered as casual contaminants of the bowel In a certain number of clinically undoubted cases of cholera examined by Anderson, vibrios inagglutinable by O Group I serum were found to be plentiful It, therefore, appeared to him that O group I sera would not pick up all choleragenic vibrios

The above evidence shows that although the majority of cases of cholera are probably due to a single type of organism, cases do occur in which other types may also be concerned.

In their subsequent studies, Taylor and Ahuja (1938-39) examined 90 strains obtained from water sources A large percentage of them agglutinated to 50 per cent or more with H and O sera Thus according to these workers, this universal distribution of vibrios in water would lead to their establishment in the human intestine and hence to their appearance in the stools of healthy individuals who subsequently acquired cholera

In a similar study, none of the vibrios isolated from water, flies, cockroaches etc. by Pasricha, Chatterjee and Das (1938) and from domestic animals by Lahiri and Das (1938) in an endemic area was agglutinated by O group I sera Pandit and Moitra (1938-39) found them in the majority of water sources examined in another endemic area suggesting wide distribution of such vibrios in nature although amongst themselves they were diverse both serologically and biochemically Thus their heterogeneity and their existence in nature in places where no cholera was occurring led Taylor (1941) to conclude that the vibrio not belonging to O sub group I does not cause cholera But all vibrios which do not possess O group I antigen may not possess the same H antigen as Gardner and Venkataraman believed, for Ahuja and Singh (1939-40), who investigated thoroughly the antigen of cholera group as a whole, found that only 35 per cent of the 219 O inagglutinable strains were agglutinable with H and O serum.

The Chemical Structure of Vibrios

Linton, Shrivastava, Mitra and Seal working during the period between 1937 and 1939 classified the vibrios on the basis of two proteins (I and II) and 3 polysaccharides (containing galactose, arabinose and glucose) into six groups These included vibrios from all parts of India as well as from other places such as Cairo, Tokyo, Basrah and Shanghai Each strain was found to contain one protein and one polysaccharide unless the strain was undergoing dissociation. The resulting classification is far simpler than those obtained by purely serological methods In general, the case and contact strains belong to groups I and II, carrier strains to groups IV and V and water vibrios to group III. Thus the water vibrios which

are according to Taylor and Ahuja are serologically heterogenous form a single chemical group III Similarly El Tor and certain strains isolated from chronic vibrio carrier in India fall into group IV; though serologically even the most refined methods have so far failed to distinguish them from true cholera vibrio while on epidemiological grounds they are to be considered harmless. Besides, when group I vibrios change their epidemiological character, they usually vary to group IV and this may be a fact of great importance (Linton, Mitra and Seal, 1938-39).

Immuno-chemical Studies of *Vibrio cholerae*

Shrivastava, Singh and Ahuja (1948, 1950) carried out protection tests in mice with vibrio polysaccharides extracted by three different methods· (i) White (1936), (ii) Shrivastava and Seal (1937) and (iii) Palmer and Gerlough (1940). The best results were obtained with extract by the phenol method of Palmer *et al.* The immunized animals were able to withstand a test infection of at least 200 lethal (L_{50}) challenge doses. The degree of protection obtained was considerable but somewhat less than the dosage of the cholera vaccine used for control tests. The fraction deteriorated on storage over P_2O_5 for 10 months.

In another study Singh and Ahuja (1950) tested the antigenic relationship of *V. cholerae* of the so-called 'A' type of vibrio (Burrows) and 'B' type of vibrios (Gallut) These workers could not support the view of Burrows and Gallut that the diagnosis of *V cholerae* should be made by the use of monospecific A serum only. Gallut's pure P_2 strain was not agglutinated by the Institute's monospecific Ogawa serum and a high titre serum raised against it did not agglutinate an Ogawa culture In the opinion of the authors the existence of pure A and pure B and C antigens could not be found in non-cholera vibrios. The reported discovery of 13 different 'O' factors in the antigenic make up of cholera vibrios was also not confirmed

Sokhey, Habbu and Bharucha (1950) examined the vaccines prepared from strains obtained from Burrows and Gallut by mouse protection test and compared the results with the vaccines prepared from the Haffkine Institute Ogawa strain. A high degree of protection was given by the vaccine prepared from the latter and N I H strains, no protection being given even by high dosage of vaccine prepared from the three Pasteur Institute strains (avirulent) and a moderate degree of protection was given by a vaccine made from the low virulent fourth strains. It was concluded that the protection power of the vaccine was not determined by the complexity of antigen structure (e.g, 13 'O' antigens) but was related to the virulence of the strain used in its manufacture

Biochemical Reactions

A large number of freshly isolated strains were tested in several enquiries in

India and reported by Taylor (1941). While all the O agglutinable strains fell into Heiberg's (1936) Group I, non-agglutinable vibrios were also found to belong to the same group. This test with the three sugars of Heiberg, *viz.*, mannose, saccharose and arabinose is not thus strictly differential.

Cholera Red Reaction

Recently Sen, Basu and Chakraborty (1946) studied the biochemistry of the Cholera Red reaction and found that the reducing agents like glucose, cysteine and *ferro-cyanide exert an inhibitory influence and the oxidising* agents acting as catalysts promote the reaction They have suggested the following modifications of the technique A suspension of 5 or 6 hours ordinary culture of vibrios is added to a sterile solution of tryptophan and sodium nitrate The addition of sulphuric acid after 45 to 60 minutes elicits Cholera Red reaction.

Voges-proskauer Test

A large number of strains tested by Taylor (1941) according to Barritt's modification and the results reported by other workers show that the majority of true cholera vibrios (non-haemolytic O group I) gives negative reaction and El Tor and non-cholera strains positive reaction With a revised technique some agglutinable strains both of the Inaba and Ogawa types gave positive reaction in varying degrees and the production of acetyl-methyl carbinol appeared to vary from time to time with the same strain (Taylor, 1938). On the whole, by means of combination of C R , V.P and sugar fermentation tests, the presumptive diagnosis of *V. cholerae* may be made within certain limits

Metabolism and Chemical Classification

The chemical changes and metabolism of 33 strains during variation were studied by Linton, Mitra and Seal (1938-39). The types of metabolism were found to coincide with the chemical groups In general, it may be said that the work on metabolism supported the chemical classification and they could be used jointly for separating vibrios into significant groups. Seal and Mitra (1939) studied the oxidation reduction potentials of 37 strains of known chemical composition and found that the curves obtained from individual organisms in each chemical group were distributed over a range which overlapped into that of another group, but by taking the curves of various groups it could be shown that the organisms containing Protein I (groups I, II and VI) had higher final potential at 72 hours than those containing *Protein II (groups II, IV and V), just as* they had a higher metabolic rate although the changes in *p*H of the media during growth were the same for all chemical groups

Haemolysis and the Position of El Tor Strains

The position of El Tor vibiros in relation to the true cholera vibrio has still remained somewhat undecided due to the variability of the haemolytic properties, although both fall into the same serological group. For haemolysis test peptone water was found unsatisfactory as some strains which gave positive results at 24 hours were negative after 48 hours Recently, Read, Pandit and Das (1942) claimed regular and better results by substituting tryptic digest broth for plain broth and making it isotonic (0 85 per cent NaCl) These workers divided vibrio strains into "Greig positive or early haemolytic" (within 24 hours) and "Greig-negative or late haemolytic" groups, the haemolysis of the former group only being specifically neutralised by the antiserum produced against "early haemolytic" group and that of the latter group being considered identical with the haemo-digestive ferments of Van Loghem Goyle (1938-39) concludes that haemolysins being thermolabile, antigenic and filterable are true exotoxin.

Pathogenicity of El Tor Vibrios

Normally in India *V. cholerae* (non-haemolytic) has not been isolated except in the immediate presence of cholera cases barring perhaps in a very few instances in the absence of cholera (Taylor, 1937, Lal, 1938, Pasricha, Lahiri and Das, 1938, Pandit and Read, 1939, Venkatraman *et al.*, 1941) while haemolytic strains have been isolated from water and healthy human sources both in the presence and absence of cholera and in some instances from actual cholera cases (Pandit and Read, 1939). The largest number of isolations has, however, been from water sources belonging to areas with different epidemiological features namely, Bengal, Bihar and Sind (Taylor, 1941) and Madras (Venkatraman *et al*, 1941) The province of Sind had been free from cholera for 10 years and yet 18 out of 775 water sources examined yielded El Tor vibrios. On the whole, the latter was considered non-pathogenic by many authorities till the occurrence of Celebes epidemic in 1937-38, 1939-40 and again in 1944, in which El Tor vibrio of serological O Group I and Heiberg's Group I, but strongly haemolytic, was directly involved

Toxin and Anti-toxin

Work done on the subject in India under the Indian Research Fund Association has not been very fruitful Various methods were employed by Ghosh (1938, 1939) in attempting to prepare toxic fractions including (a) the use of filtrates from media of different reactions and with the addition of carbohydrates, (b) filtrates of autolysates and (c) filtrates from suspension treated by freezing and thawing or after tryptic digestion etc. and the toxic material obtained by the above methods produced reaction of the nature of protein shock In this respect very little difference was noted between the cholera and so-called non-cholera vibrios, though the latter

in some cases proved more toxic The anti-toxins produced against these toxins failed to protect animals although *in vitro* neutralisation could be effected in some instances The intradermal reaction given by these products were also non-specific. Banerjee (1942) obtained toxin from a thick vibrio culture by dialysis through cellophane bags, the intraperitoneal and I V. m l.d s of the filtrate being 0.25 ml and 1 ml respectively. Basu, Chaudhury and Basu (1940) studied the fluid diffusate of *V. cholerae* culture in proteose peptone solution. It contained carbohydrate but no protein, was toxic to white rat but not to guinea-pigs, and could be used to differentiate true cholera vibrio from paracholera and saprophytic types on the basis of invasiveness and toxicity of these organisms when injected into guinea-pig.

Variation and Dissociation of Vibrios

(a) *Chemical and physical basis of variation* Various workers from time to time have observed and described variations in the vibrio characteristics Starting from a single cell culture Linton, Seal and Mitra (1937-38) showed that the smooth-rough transition was accompanied by changes in salt-stability, serology, metabolic activity and chemical constitution, but the variation remained strictly limited within the six chemical groups propounded by them These workers (Linton, Mitra and Seal, 1938-39) also showed that the change brought about by the treatment with activated antisera led to an increased surface potential in the survivors Organisms which were found quite distinct in electrophoresis in the smooth state often became similar or identical in this respect when grown in the rough state This finding gives the physical basis of Bruce White's findings that rough vibrio strains are more generalised serologically than the smooth strains The metabolic activities of these variants were also found to correspond to the new groups into which they now fell

Shrivastava and Seal (1937) obtained highly specific polysaccharide which gave precipitin reaction at a titre of 12 million dilution against the homologous antisera Using the same chemical method Linton, Shrivastava and Seal (1937-38) studied the character of the polysaccharide which a single strain produced when grown in 8 different liquid media containing various combinations of peptone, infusion broth, glucose and buffers. The results showed that the type of medium had a profound effect upon the yield and quality of specific polysaccharide.

Again, Linton, Shrivastava, Seal and Mookerji (1938-39), while studying the specific polysaccharides of strains belonging to different chemical groups, noted the presence of a lipoid containing polysaccharide in the strains isolated from the earlier part of the epidemic, which was absent in strains isolated in the latter part of the epidemic and in strains maintained for a long time in the laboratory or isolated from water and carriers. This lipoid portion dominated the specificity of the organisms which led the authors to suggest a bearing of this special structure

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of vibrios of various types through house-flies, bred in the laboratory in a sterile manner, resulted in changes in chemical structure and metabolic activity, but not in the fermentation or O-agglutination reactions. Soparker (1938), on the other hand, could not obtain survival of vibrios in the flies longer than a few hours except in a few instances and they found that the extracts made from the intestine or abdomen of the fly had vibriocidal effect

(e) *Intestinal flora and variation* . Fairs and festivals have been a potent source of cholera outbreak in India. How the first case arises in such a situation remains very often a matter of speculation. Napier and Gupta (1942) noted that low or absence of gastric acidity allowed the vibrios to pass into the intestine, while Ghosh and Mukerjee (1941) showed the presence of *B. coli* autolysate in the cholera stools and the corresponding antibody in the sera of convalescent cholera patients. In their views the absorption of the autolysate might play a part in aggravating the symptoms (symbiotic action) Out of 2,000 non-cholera patients Pasricha, Lahiri and Das (1938) isolated typical non-haemolytic Inaba O-positive vibrios from three individuals with history of (1) ill-defined abdominal symptoms for 2½ years before admission, (2) chronic diarrhoea on admission and (3) dysentery for 6 months prior to admission

Viability of *V cholerae* under Natural and Artificial Conditions

Napier and Gupta (1942) from their study of the effect of gastric juice on the survival of *V cholerae* concluded that individuals with low acid gastric juice might be much more susceptible to cholera infection than the individuals with a high or normal acid content

Read, Singh, Seal and Bose (1939) in their study of the conditions required for the growth and survival of *V cholerae* in natural waters found that both salt and organic matter were necessary for the multiplication and survival of cholera vibrio In artificial water without salt, vibrios died within 24 hours The optimum salt concentration at which there was good growth was 2 per cent at *p*H 9 2 but this amount could be decreased with the increase of organic matter. In the presence of inagglutinable vibrio the agglutinable vibrio could survive for 2 weeks and other organisms in comparatively equal inocula did not prevent multiplication of *V cholerae* up to 5 days or little longer. These findings suggested that survival of cholera vibrio in water was not a proved factor in the maintenance of cholera endemicity The period of survival of freshly isolated vibrio tested by Lahiri, Das and Malek (1939) in the untreated, autoclaved, filtered, and filtered and autoclaved and filtered samples of raw water from different sources varied between 1 hour and 18 days, the longest period being shown by the filtered and autoclaved and filtered samples.

Action of Antiseptics on Vibrios

Panja and Ghosh (1943*b*) found 1/5000 permanganate to kill vibrios in 5 minutes in the presence of organic matter but this was not supported by other workers. The same authors (1943*a*) tried the effect of different dyes on vibrios and found 1/10000 brilliant green to exert a complete selective bactericidal effect isolated from the Hooghly river. Brilliant green at final dilution of 1/5000 also killed cholera vibrios in stools The same authors (1945) studied the viability of *V. cholerae* in *dahi* (curdled milk), lactic, hydrochloric, acetic and citric acid with adjustment of *p*H at 4 4 The vibrios were killed in *dahi* within 5 minutes and all acids were highly vibriocidal except citric acid. Lime juice at *p*H 4 4 takes ½ hour to kill vibrios and the undiluted (*p*H 2.8) only 5 minutes.

Bose and Chakraborty (1948) studied the bactericidal action of metallic copper on *V. cholerae*. Even in the presence of organic N 0 4 mg per 100 cm^3 the vibriocidal effect was complete within 1½ hours but the presence of copper could not be diluted by chemical test. No effect was, however, seen if the water treated with copper foil for 48 hours was used for the preparation of the media. The lethal effect was also seen if a polished copper vessel was used instead of the foil

Growth Medium for *V cholerae*

Bose (1939*a*) obtained the best growth of *V cholerae* in papain-digested mutton broth prepared by digesting 300 g of mutton with 5 g of papain for 2 hours at 60°C Vardon and Dutta Roy (1938), on the other hand, found better result with more cheaply prepared papain digested casein broth which could be used for the culture of bacteriophage as well as for general laboratory use. Casein hydrolysate broth introduced by Seal and Mookerji (1940-41) in plague work has been adopted by Sokhey (1944) for the preparation of cholera vaccine and by Shrivastava (1948) for vibrio polysaccharide studies

Pasricha, Panja and Paul (1940) found it more convenient to adopt a "dilution method" for the isolation of pathogenic bacteria from faeces, the dilution being made up to 1 · 100 million or more. Later, Panja (1942) could obtain 87 per cent positive growth of vibrios (non-differential) by this method as against 44 per cent by direct bile-salt agar plating, when a small amount of stool was mixed with peptone water and partly sucked through L_3 candle placed into boric-peptone water at *p*H 9.0.

Venkataraman and Ramakrishnan (1941) introduced a buffered sea-salt solution at *p*H 9 2 as a preserving medium for *V. cholerae* and this greatly facilitated the isolation of vibrios from long distances, although ordinary sea-salt mixture at *p*H 9 2 used by Seal (1940) was also quite good.

A simple medium consisting of salt mixture, 1-cystine, glucose and marmite at *p*H 8 8 for the cultivation of *V cholerae* was suggested by Veeraraghavan (1949). The salt mixture contained ammonium sulphate and $NaHCO_3$ and glucose were added at 10, 16 and 24 hours to keep the *p*H above 8.0. The concentration of vibrios in the culture reached 12-14000 million per mm[3] at the end of 30 hours. Sokhey *et al* (1950*b*) found a modified casein hydrolysate broth without treatment with Tween 80 at a N concentration of 135 mg per 100 ml distributed in 500 ml in three-litre-Haffkine-flask gave good growth of *V cholerae* — yielding a vaccine of higher potency than usual.

Differential isolation of V cholerae Experience showed that alkaline peptone water ordinarily used for isolation of vibrios from water and stools allowed growth of many other organisms which were prejudicial to their own growth. Read (1939) surmounted this difficulty by introducing a modified Wilson and Blair's bismuth-sulphite enrichment medium (at *p*H 9 2) originally prepared for the isolation of *B typhosus*. Seal (1940) made further modification of Read's mannose-bismuth-sulphite medium and compared its efficiency with that of alkaline peptone water at *p*H 9.2 in the isolation of *V cholerae* from human as well as non-human sources. The results showed some difference in favour of this new medium over alkaline peptone water This medium has now been adopted for the differential isolation of *V cholerae* by different laboratories in India In this connection Bose (1939*b*) prepared unpurified mannose solution from ivory-nut shavings, which was found equally good as the pure product.

Cholera Bacteriophage

Moitra (1939) in an extended study on the inhibition of cholera bacteriophage by vibrio extracts together with their precipitin reactions of these extracts could divide the vibrios into two main groups, viz., (a) typical smooth cholera and El Tor vibrios belonging to O sub-group I, and (b) a heterogenous group of water and other vibrios The inhibition and precipitin reactions seemed to depend upon a common factor related to the complex polysaccharide type as determined by chemical analysis

Pasricha and Paul (1941) isolated cholera bacteriophage from many samples of garden and field soil up to the level of 3 ft. below the surface. Pasricha, Lahiri and De Monte (*1941*) *with the application of reciprocal cross test found that the* LL phage described by Bruce White behaved as a new phage which the author described as N cholera phage and which like A phage acted on *V. cholerae* alone.

Pathology of Cholera

Attempts to isolate *V. cholerae* from urine of 122 cholera cases by Chatterjee and Malik (1938) and from blood of 36 cases by De Monte and Gupta (1938)

were unsuccessful; while Pasricha, De Monte and Chatterji (1939) succeeded in isolating a typical cholera vibrio from the liver puncture of a young boy who showed symptoms of hepatitis with jaundice as a complication of cholera but the latter ultimately survived. Banerjee (1939*a*) described the pathology of cholera. He found extreme necrotic changes in the intestinal epithelium, distension of the capillaries, sub-epithelial oedema and distension of gland tubules with cholera vibrios. Thymus was almost invariably enlarged but not the spleen and degenerative changes were noted in the suprarenal cortex. On the clinical side, two types of cholera were distinguished, viz., "renal failure type" and the "vasomotor failure type". In the former, hypochlorimia was of greater importance than dehydration while in the latter profuse evacuation followed increased permeability from the intestinal vessels and the nature of gastro-intestinal allergy probably due to increased absorption of histamine from the intestine causing dilatation of the blood vessels and fall of blood pressure. In a further study on the subject the author (Banerjee, 1941*a*, *b*) concluded that hypochlorimia led to dehydration, reduction of nitrogenous waste products and renal failure and that the renal vascular failure in cholera was only a part of the whole systematic peripheral capillary failure This capillary failure in the kidney in conjunction with great loss of interstitial fluid and hypochlorimia constituted the mechanism of renal failure in cholera.

Chatterjee (1939*a*, *b*, *c* 1941, 1946 and 1947) who made more extensive studies on the pathology of cholera noted only congestive changes in the intestinal epithelium with enlargement of lymphoid follicles, dilatation of capillaries and sinusoides, preponderance of eosinophiles (15 to 20 per cent) in the red bone marrow and enlargement of spleen (unlike Banerjee's findings). The shock in cholera according to him did not depend upon the dehydration alone but also upon great dilatation of capillaries due to an allergic state Most of the congestive changes in the kidney such as, congestion and swelling of glomeruli was also ascribed to the action of histamine-like substance and the anuria to deficient blood pressure.

In the above description, the symptoms of cholera have been sought to be explained by the production of histamine in the intestinal canal (Chatterjee, 1939*c*) but all things considered, however, it seems probable that the effects of the cholera vibrio are due to specific toxins elaborated in the intestinal wall.

Pasricha, Chatterji and Paul (1939) tested the agglutinin contents of cholera patients and found that agglutinins usually develop by the fourth day but were more frequent and of high titre by the seventh day and that the H agglutinin appeared earlier and was more consistent and of higher titre than O agglutinins Yacob and Chowdhury (1945*a*) found agglutinins to appear in cholera cases as early as the second day becoming maximum (O titre — 1 : 150) by the sixth day with the possibility of persisting for 3½ months or more and hence retrospective diagnosis was possible.

Biochemical Studies

Pasricha and Malik (1940) examined the blood of acute cases of cholera before saline transfusion and found wide variation in its different constituents. Generally, there is (1) an increase in the cell volume and haemoglobin percentage directly proportional to the cell volume, (2) a decrease in the moisture content of the blood and plasma, (3) an appreciable increase in the urea and non-protein nitrogen, total plasma proteins, fibrin and globulin fractions, (4) an increase in organic phosphates, (5) an appreciable increase in the glucose concentration of blood and plasma and (6) diminution in the concentration of sodium chloride in the blood and plasma Chatterjee and Sarkar (1941) carried out similar studies, the additional findings being diminution of sodium content and increase in potassium content, lowering of serum calcium, diminution of serum chloride proportionately less than that of sodium, decrease in blood sugar (contrary to Pasricha and Malik's finding), increase in inorganic phosphates and marked decrease in alkali reserve (acidosis). In an extended study Chatterjee, (1946) found a high rise in the free, total and combined phenol in the blood of undoubted cholera cases, the respective averages being 4.43 mg, 1.38 mg and 5 60 mg per 100 ml of blood against the normal averages of 2 24 mg, 0 39 mg and 2 63 mg respectively The specific gravity of blood falls to normal with saline transfusions, but the increased phenol persists for sometime.

De Monte and Gupta (1941) found the sedimentation rate of blood increased in 53 out of 79 cases examined but the increased specific gravity might also be responsible for it The chemical constituents of the stools of cholera patients were studied by Ghosh and Chakraborty (1940). There is high elimination of alkaline base and chlorides in the cholera stools leading to acidosis and disturbance of osmotic balance and suppression of urine

The amount of sodium chloride present in the cholera vomit varied from 66 to 321 mg per 100 ml and bore no relation to the H-ion concentration which ranged between 5 0 to 7.5 (Panja, Malik and Paul, 1942).

Epidemiology of Cholera

Lal, Raja, Swaroop and Basak (1941) defined the endemicity of cholera, the chief areas being comprised of Assam, Bengal and Orissa. The Bengal districts, however, presented considerable heterogeneity in regard to their cholera experience and there were evidences of heterogeneity within the district themselves. Taking the thanas as units for sorting out homogeneous cholera districts in terms of mean incidence and its variability the areas were redistributed into homogeneous cholera districts which did not correspond to the existing political boundaries The net endemicity was determined by the method of partial regression to eliminate factors, such as, differences in population, area and number of thanas

to a district and the homogeneous districts classified into various grades of endemic and non-endemic areas This study was extended by Raja (1942) to certain districts of south India, where inspite of the six-yearly periodicity the mortality of cholera seems to have declined between 1930-1941.

The Punjab, according to Yacob (1944), is a non-endemic cholera area, the main reasons adduced for the continuance of cholera are the habits of the people, the prevalence of massed pilgrimage and probably also the climatic environmental factor, but all these factors are capable of being altered, regulated and countered by appropriate measures and the author obtained a signal success in 1941, "Solar eclipse" fair at Thanessar (Kurukshetra) which passed off without a single case of cholera, the most important measure taken being the protection of pilgrims to and from the fairs by inoculation, besides the usual environmental sanitation.

Yacob and Chaudhuri (1945*b*) tested aerated drinks treated with ice artificially contaminated with cholera vibrio before the water was converted into ice. They contended that given sufficient time, an aerated drink even if infected with cholera vibrio would become harmless and similarly cholera-infected water when converted into ice and served in drinks might not prove harmful.

Seal (1945) discussed the problem of endemicity of cholera in Bengal which remained unsolved in spite of the various new researches on the subject of cholera. In intensive field studies in an endemic area in Bengal true cholera vibrio defined by Gardner and Venkataraman could not be isolated from the stools of the general population or from water except in direct relation to the cholera cases, the transmission mainly occurring from case to case, while close contact carriers and waters being considered as infective agents for short periods and at short range He, therefore, put the question "where and how does the cholera vibrio exists before a case occurs in the endemic area?" and in this connection he emphasized the importance of the study of the relationship of other types of vibrio found in human beings and water sources etc. to the so-called true cholera vibrio.

Rao (1947) in Hyderabad State availed of an opportunity to follow the events in a moving religious fairs (*Palkies*) which culminated in an out break of cholera He noted that the cholera cases might occur at the commencement of the journey, be present in villages on the way and be brought back with their return journey from the shrine of attendance. In this particular pilgrimage occasional cases of cholera occurred on the outward journey but it did not spread and no cases occurred in the return journey. This is claimed to have been achieved by the following precautions, viz., compulsory inoculations of all pilgrims, treatment of wells with bleaching powder, great care over feeding of the pilgrims which was the obligation of the villagers en route

TREATMENT OF CHOLERA

(1) *Bacteriophage* Chatterji and Deo (1938) held that phage was mainly concerned in bringing about the cure of the patient Although the dominant phage used for the treatment of cholera was the A type, phage mixture was issued by the Bihar Government for therapeutic purposes and administered in massive dose at the earliest opportunity Simultaneous comparison of different methods of treatment, viz, (i) divided doses of calomel, (ii) potassium permanganate, (iii) essential oils, (iv) bacteriophage and (v) M & B 693, was made by Pasricha, De Monte, Chatterji and Mian (1939) The least fatality (4 5 per cent) occurred among the phage treated group The authors, therefore, advocated the use of bacteriophage as a routine measure in the treatment of cholera Mitra (1938-39) supported this view from his long experience of its use in Bihar both as a curative and prophylactic measure

(2) *Intravenous saline* Banerjee (1938) from his experience in 1714 intravenous injections of salt solution in 1,000 cholera patients noted rigor in 81 per cent cases following I.V saline He found it advisable before beginning the saline injection to reduce the high rectal temperature by an enema of 15 to 20 ounces of ice-cooled normal salt solution, as in this way the reaction appeared to be diminished in intensity. Pasricha, Malik and Paul (1941) as well as Panja, Malik, Paul and Ghosh (1942) showed the importance of pyrogen-free distilled water in the preparation of material (saline, etc) for parenteral administration. Paul and Chatterji (1944) observed that the pyrogenic reaction could not only be induced by the presence of pyrogenic substances in the distilled water but also if the solutions were more acid or alkaline than the blood can buffer Bose and Ahuja (1944) described a biological method for detection of pyrogens in fluids.

(3) *Chemotherapy* Although "sulpha" drugs have not yet proved specific for the treatment of cholera before its advent there was no other drug worth mention which showed any better vibriocidal or vibriostatic action

Sulphaguanidine In a preliminary trial Chopra, De Monte and Chatterji (1941) obtained encouraging results even with low doses, the fatality in 218 cases treated with this drug being 3 4 per cent as against 6 38 per cent in a series of 94 controls treated with saline perfusion only Misra (1944) who compared it with other treatments, viz, divided doses of calomel, bacteriophage, and sulphapyridium found sulphaguanidine to give the best result with an initial dose of 5 g followed by 2 g every 6 hours, only one death occurred out of 16 cases tried Lahiri (1945) in a comparative study of the efficiency of sulphaguanidine, sulphathiazole and calomel in the treatment of 315 patients noted the fatality rates as 14 9, 28 and 29.5 respectively. The initial dose of sulphaguanidine advocated was 2 g followed by 1 g 2 hourly twice and then at 4 hourly intervals, the total dose in 24 hours being up to 6 to 7 g He also found "encortone" beneficial when given with

glucose intravenously before saline transfusion. The above results of sulphaguanidine in hospital patients were confirmed by Gupta, Chatterjee, Paul and Ghosh (1945) and Pasricha, Paul, Das Gupta and Das (1947*a*, *b*) in much larger series of cases, viz., 525 and 2,288 respectively including the controls. The doses used were 5 g on admission and 2.5 g every 4 hours to reach a total of 20 g in 24 hours The death rates amongst sulphaguanidine treated cases in the treated groups being 1 1 and 3 7 per cent as against 5.0 and 7 5 per cent in the controls. No cases treated with sulphaguanidine developed uraemia and the drug was also found both vibriostatic and vibriocidal (low-grade) *in vitro*.

Seal (1947) tried sulphaguanidine in 290 cases under field conditions in which most of the cases were seen in their early stages with very good results, the death rate among the drug treated cases being only 1.5 as against 43.5 per cent among the untreated or otherwise treated controls The initial dose was 3 g every 3 hours until stools were reduced in number, when the dose was gradually reduced and given at longer intervals The author considers that the drug may be safely kept in villages and administered generally in suspicious gastro-intestinal diseases "as an emergency or first aid measure"

Among the other drugs used were sulphasuxidine and sulphadiazine and phthalyl sulphathiazole by Pasricha, Paul, Das Gupta and Das (1947*a*, *b*), sulphadiazine and sulphaguanidine by Lahiri (1948) and a new compound 6257 (formo-cibazol) by Bhatnagar, Fernandes, De Sa and Divekar (1948*a*, *b*) Both sulphasuxidine and phthalyl sulphathiazole did not show significant differences in death rate among the treated cases and the controls. Sulphadiazine and sulphaguanidine gave almost equal results in the hands of Lahiri (1948) who considered both as beneficial in cholera cases In the treatment with the above drugs saline was freely used to combat dehydration whenever needed. However, with the new compound formo-cibazol Bhatnagar *et al.* (1948*a*, *b*) claimed very good results in the field trial, the death rate being only 4 per cent in 85 cases treated. The drug is also vibriostatic *in vitro* and vibriocidal *in vivo* (animal experiments). The drug is administered in 10 g doses on the first day, two doses of 4 g on the second day and two of 1 g each morning and evening every subsequent day for 5 days No toxic effect was seen even with 50 g in 24 hours.

Sen and Basu (1945) found that sulphanilyl benzamide in 1 20,000 exerted no action on cholera vibrio but it acts on vibrios which are not lysed by bacteriophage and thus the lytic action of choleraphage is synergistically increased in the presence of this drug and it has been suggested that a combination of the two might prove useful in the treatment of cholera.

Atebrin and quinacrine: Panja (1945*a*) treated twenty human cases of cholera with atebrin in doses of 1 tablet (presumably 0.1 g) every 15 to 30 min until 4 to 6 tablets had been given in one day. Some patients received two more tablets

on the following day. These patients who are admitted late in the collapsed state also received hypertonic saline on admission All cases except one survived. Results with quinacrine were not encouraging.

Antibiotics in the treatment of cholera Chaudhuri, Ghosal and Rai Chaudhuri (1950) treated 10 patients with chloromycetin against the same number as control. 2 patients died in the treated group and 1 in the control, but those who survived became vibrio negative in 48 hours The authors concluded that to be of value the drug should be given early before severe dehydration developed, and suggested prophylactic use of the drug

Value of Prophylactic Inoculation

Millar and Mohiuddin (1937) inoculated 13,06,273 persons during an epidemic of cholera in Kashmir in 1935, in which 9,304 cases occurred with 4,449 deaths Among 1,30,713, 15,825 and 21,871 persons receiving 0.5 ml, 1 ml and no vaccine respectively and exposed to risk the incidence of cholera was 0.29 and 11.35 per cent respectively, which certainly went in favour of the vaccinated over the non-vaccinated

A large scale study of the value of cholera inoculation was made in 2,350 villages in South India by Adiseshan, Pandit and Venkatraman (1947). Among 7,09,977 protected persons in the inoculated population and 21,19,568 un-inoculated persons the incidences of cholera were 1 57 and 16 20 per 1,000 respectively ($X^2 = 9135\ 09$) In the second and subsequent outbreak 6,580 cases of cholera occurred among the un-inoculated and only 241 in the inoculated group, *i e*, 14.2 times greater in the unprotected group than in the protected group Thus, it is confirmed that previous inoculation confers immunity and according to the present observation it lasts at least 6 months to one year

Chandrasekhar (1947) made a searching statistical analysis of the efficiency of the above anti-cholera inoculation in 63 cheris of the Arcot district The attack rate in the un-inoculated, according to this analysis, was found to be 2 4 times that in the inoculated population and although the average resistance of the population increased by inoculation there was no significant difference between the case fatality rates among those attacked in the inoculated and un-inoculated population This was the first wide scale study based on strict statistical analysis which proved the efficiency of cholera prophylactic inoculation Panja and Das (1947) produced immunity in cholera by intradermal inoculation of cholera vaccine. Seal (1948) says that the intradermal inoculation is of great value and useful for cholera inoculation programme.

Pasricha, Chatterji and Paul (1938) examined 14 samples of vaccine, both local and foreign made, for (1) sterility, (2) freedom from abnormal toxicity, (3) antigenic response in rabbits, (4) antigenic response in man, and (5) protection values

in guinea-pigs. All the six cholera vaccines from the recognized laboratories gave satisfactory antigenic response and protected guinea-pigs against 2 M.L.D.'s of *V. cholerae* Four of the eight commercial preparations gave uniformly negative results.

Ahuja and Singh (1948) studied the response of human volunteers to inoculation with vaccines prepared from different forms of *V. cholerae* by estimation of rise in bactericidal titre of their sera and the value of the sera for passive protection of guinea-pigs injected with mucin. Bacteriolysins against both homologous and heterologous forms were found to have been developed by inoculation with either of the pure-form vaccines or mixed vaccines, the highest level reaching on the 8th day and practically disappearing with 6 months The passive protection test was, however, found more sensitive and the sera obtained by injection of mixed vaccine gave the best protection From active immunization of guinea-pigs with these vaccines and from the results obtained the authors concluded "An Inaba strain vaccine affords just as good protection against Inaba infection as against Ogawa infection and *vice versa*."

Preparation of Cholera Vaccine

The Cholera Advisory Committee of the Indian Research Fund Association lately made the following recommendations which are in accordance with the practice in the majority of laboratories in India.

" The vaccine should consist of suspension of the vibrios obtained by washing off the growth from a 24-hour agar culture with 0 85 per cent saline solution The vibrios should be killed by the addition of 1 per cent phenol to the suspension without the application of heat The phenol should be reduced to 0 5 per cent in the vaccine finally issued The vibrio content of the vaccine should be stated, and it is recommended that when the vaccine is likely to be used for single dose inoculation of 1.0 cm^3 its strength should approximately be 8,000 millions per cm^3

The strains of vibrio used should (1) be smooth and translucent, (2) form stable suspensions in normal salt solution, (3) have the serological characters of Group O No. I Gardner and Venkatraman sub-type Inaba and Ogawa and agglutinate to a significant titre with serum prepared from the dried Inaba and Ogawa 'O' antigens provided by the Standards Laboratory, Oxford, (4) ferment mannose and saccharose but not arabinose, (5) not be haemolytic, (6) be isolated from cases of cholera during an epidemic, (7) be highly stable even in subculture

Sokhey's cholera vaccine Sokhey (1944) prepared cholera vaccine in the casein hydrolysate fluid medium originally adapted from Mullar and Johnson's (1941) medium by Seal and Mukherji (1940-41) for the cultivation of plague organism. The organisms are grown in this medium for 72 hours at 37°C and then killed with formalin (0.1 per cent) and preserved in phenyl mercuric nitrate. This vaccine

tested biologically in white mice has been claimed to be superior to agar grown vaccine. Venkatraman (1945) reported favourably upon this vaccine as tested in mice but the final opinion should await some controlled observations in the field.

Biological assay of cholera vaccine. A method of biological assay of the protective value of cholera vaccine has been developed by Sokhey *et al.*, (1950*c*) and is in routine use in the Haffkine Institute, Bombay Groups of mice are given graded doses of vaccine under test and are then submitted to challenge with a standard dose of a virulent strain of *V. cholerae* (0.5 cm³ of 5 per cent much in containing 1,00,000 *V. cholerae*) The mean dose of vaccine giving 50 per cent protection is taken as the measure of potency

Control of cholera Seal (1946 and 1948) studied a large number of cholera outbreaks in certain rural areas in West Bengal and recommended certain short and long term measures for the control and prevention of endemic cholera in the rural areas of Bengal. The temporary measures were designed mainly to control the outbreaks while in force The importance of early notification was actually demonstrated and acid mixture and sulphaguanidine were used as drug prophylaxis In fact, the author found it a wise policy to avoid inoculation of the members of the affected family for at least 3 days, during which period he obtained very good results by administering acid mixture and sulphaguanidine along with disinfection and other measures

The three new features suggested as long term measures for the control and prevention of cholera in rural areas in Bengal were (1) the method of anti-cholera revaccination by intracutaneous inoculation of one-tenth cm³ of the usual dose, (2) 'segregation cottage' for every village, built and run on voluntary basis, and (3) a "special epidemic control unit" consisting of a field and a laboratory section for each subdivision of Bengal, which can work efficiently only when the communication is improved.

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SALMONELLA AND DYSENTERY GROUP OF INTESTINAL PATHOGENS

On the Isolation of the Pathogens :

Selection of media for the isolation of intestinal pathogens gained importance in the hand of Panja and Ghosh (1943). D E.C. medium evolved by them is claimed to be superior to MacConkey, S S Agar and Willson-Blairs' medium for isolation of enteric, dysentery and cholera group of organisms From examination of sewage water, Panja and Ghosh (1945) concluded that D E.C. medium was superior to other standard medium for isolation of *S. typhi* Panja (1942) described a novel method for the isolation of *V cholerae* Stool mixed with little peptone water was first put into a L_3 candle filter and then placed dipping in a boric (0.08 per cent) peptone water at *p*H 9.0 A little of stool mixture was drawn through suction and the whole unit as such was incubated for 24-48 hours He claimed that by this procedure he could obtain pure culture of vibrio as the organism could readily grow through the pores of the filter into the outside medium. Basu, Sen and Sengupta (1945) used papain digest of groundnut meal powder in place of meat and showed that this media supplemented by beef liver digest, would support the growth of intestinal bacteria From comparative study of sapenin broth and bile broth as a primary culture medium for culture of the enteric group of organisms, Soman (1946) demonstrated that bile broth would give better result. Pasricha, Panja and paul (1940) advocates serial dilution method in seeding the plates in primary culture of steel for pathogenes. They feel by this method in practice discreet colonies could be obtained for study. Pandit, Sanjiva Rao and Shortt (1938) inoculated chorioallantoic membrane with *Salmonella typhosus* and produced lesions which could be reproduced with filtrates of the ground lesions. No satisfactory explanation of the latter observation was, however, offered

On the Sero-Immunology of the Infection .

Bhatnagar (1938*a*) isolated a smooth, non-flaggellated strain of *Salmonella typhosum*, which contains large quota of Vi antigen, minimum amount of O antigen and no H antigen He stressed the importance of agglutination reaction with this strain, since use of this in the agglutination test precludes previous adsorption of H and O antibodies — tedious procedure in routine technique. He further supported conclusion of Felix — that Vi agglutination test is particularly valuable in detecting the carrier state Later on Bhatnagar, Speechly and Singh (1938) showed that Vi strain had a higher specificity for Vi antiserum but less so with O antiserum. Authors also described some morphological peculiarities of such strain, the bacilli almost look coccal in shape surrounded by capsules or capsular zones with tendency to occur in large or small groups resembling the arrangement of staphylococci. Seshadrinathan and Pai (1940) demonstrated that Vi test is superior to H agglu-

tination in the diagnosis of typhoid fever. Interesting study on somatic antigen of *Salmonella typhosum* was made by Roy (1943) and Mukerjee (1944) The former obtained a fraction by acetone precipitation which gave positive Molic and Buirett tests but negative Million's and Esbach's. The precipitate reacted with pure O antiserum and was lethal to mice in a dose of 0.25 mg, immunizing potency was reported to be of high order. The latter made an *in vitro* and *in vivo* studies of somatic antigens of salmonella type against various reagents as it would happen to encounter when administered orally. Acid-pepsin and gastric juice destroyed H antigen but did not affect O antigen Vi antigen was reduced in potency. *In vivo* study also corroborated authors' *in vitro* data.

A study in prognostic evaluation of typhoid fever by laboratory investigation was made by Bhatnagar (1944) An early rise in Vi titre is common in milder infection and reasonably high sustained titre indicates manner of recovery Unfavourable prognosis is indicated by very high O titre Absence of lack of appropriate rise in agglutination titre does not exclude typhoid infection.

Other Group of Salmonellosis ·

Not much work has been done on other salmonellosis Shirlaw, McDonald and Hayes (1945) isolated a strain of canial salmonella which behaved biochemically and serologically identical with *S typhi murim* Hayes and Freeman (1945) made a survey of salmonellosis in the Army stations in India from 1941 to the beginning of 1945 Apart from *S typhosum*, *S*! *paratyphosum* C and *S. enteritidis* produced bulk of infection during that period On the basis of their data that this two strains be included in the vaccine in routine use for the prophylaxis of the enteric group of fevers in India. Ghosal (1941) contributed a paper on salmonella infection in rats in Calcutta. He demonstrated that most rats were infected with *B. typhi murium* and less by enteritidis'

Antigenic study on other Salmonella has also been done by Freeman (1947) who isolated a strain of Salmonella poona from a guinea-pig (1948)

On Bacillary Dysentery ·

Work on the microbiology of dysentery infection had been scanty during the years under review, though this infection is not less important amongst gastro-enteric ailments in the Indian population Goyal (1948) isolated exotoxin and endotoxin from *Shiga bacillus* and tested their toxicity. M L.D. of extracted exotoxin was 1.5 mg while that of endotoxin 140 mg. Postmortem findings were similar in both None of them caused diarrhoea in monkeys, rabbits or mice.

Forsyth (1942) prepared dysentery vaccine with flexner and shiga strains and showed a fairly good amount of protection in mice and monkeys against experi-

mental infections with live vacilli. He recommended by virtue of his data a field trial of the vaccine prepared on his lines.

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B. COLI AEROGENES GROUP

Most of the work on this group of microbes has been carried out with the intention of using the bacteria as a criterion for estimating the hygienic quality of water supply Authors, to be mentioned, on the score are Raghavachari and Iyer (1939). According to them the only reliable index for portability of India waters is revealed by coliform count and the recency of pollution in water supplies cannot be estimated by resistance of these organisms to sunlight and storage It appears that *B coli* communi when stored in water under natural conditions does not disappear as quickly as one believed Specimens of fresh stools from fifteen healthy human subjects on culture showed in 60 per cent of cases presence of *B aerogenes*. Krishnan and Chawla (1941) examined stool of intestinal disorders and isolated *B aerogenes* in 35.3 per cent cases of unknown aetiology and in 90 per cent of typho-cholera cases. The types of coliforms present in the faeces examined were arranged in descending order of frequency, as follows

Bact Coli, Type I	=	77 per cent.
Bact aerogenes, Type I	=	15 per cent
Bact coli, Type II	=	3 per cent.
Irregular types	=	2 per cent.
Bact aerogenes Type II	=	1 per cent
Intermediate types	=	1 per cent.

These observations would suggest that *B aerogenes* should also be considered as an indication of faecal pollution Seshadrinathan and Venkataswami (1943) made a study on 72 coliform bacteria isolated from infections of urinary tract and showed that 30 per cent were caused by aerogenes and 35 per cent by coli group Intermediates accounted for 18 per cent while the rest could not be grouped.

Banerjea and Sen (1940) have shown the Eijkman test carried with double strength Eijkman medium would give significantly better values for positive results obtained with coliform organisms from human faeces, which could be further bettered, according to them, with double strength MacConkey broth incubated at 44°C.

Modification of Voges-Proskauer test has been suggested by Iyer and Raghavachari (1939), Banerjea (1944) and also by Panja and Bose (1945).

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PROTEUS-PSEUDOMONA GROUP

Nutritional requirements of *Ps pyocyanea* were studied by Pandalai and Rao (1942) employing 10 strains The authors described two synthetic media — one for growth and the other for growth and pigment formation According to them, optimum *p*H for growth and pigment formation was 7 4 Phosphate was found essential for growth but sulphur and lactic acid for pigment formation A lot of pigment was produced even in the protein-free synthetic media. Magnesium was not essential either for growth or for pigment formation

Bose and Ahuja (1944) made an attempt to standardise the test for the presence of pyrogen in fluid used for intravenous transfusion According to them pyrexia in rabbits following administration of all such fluid was a better index of pyrogen estimation than the leucopenia which also happen to occur after injection of pyrogen

Pandit, Rao and Shortt (1938) described experimental lesions in the chorio-allantoic membranes of growing chick embryo when inoculated with *Bacillus proteus* X 19 The lesions were not dissimilar to those met with in known virus infection and were capable of transferring infection in series even when the filtrate of ground lesions (through gradocol membrane) were used for reinoculation The question whether such successful passage through filtrates was due to filtrable forms of microbe still remains unanswered

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PLAGUE

Position of plague in India

During the period under review 'Plague' has not presented as much problem as it did during the pandemic in the earlier years of the present century. The approximately yearly average of deaths has been 1898-1918 = 500,000; 1921-30 = 1,00,000; 1931-35 = 50,000, 1939-48 = 29,500. It is now practically confined to U P., Bombay, Bihar, C.P., Madras, Punjab and Hyderabad and Mysore States, the other States being free or almost free from this disease. It reappeared in Calcutta in 1948 (Lal and Seal, 1948), after a virtual absence for about 25 years from the city and nearly 700 cases occurred till 1951 after which no human case has been recorded Nevertheless, researches in plague in India during the decade were mainly directed towards the study of the organism and improvement of prophylactic vaccine, chemotherapy and control measures

1 Study of the organism

(a) *Nutritional requirements and growth medium for* P pestis. Ordinary nutrient agar was not found suitable for the growth of virulent plague bacilli. Sokhey (1939*a*) introduced 5 per cent rabbit's blood agar as the medium of choice, the optimum temperature of incubation being 37°C for 48 hours. The optimum temperature of incubation for the growth of this organism in the liquid medium, nutrient broth was found by Sokhey and Habbu (1943*a*) to be 27°C at *p*H 7.2 to 7 6.

Very interesting work has been done by Rao (1939, 1940) in the field of nutritional requirements His findings are (1) three amino-acids proline, phenylalanine and cystine are indispensable while the presence of glycine though not essential, is stimulatory; (2) accessory growth factors have no essential role but haematin was found highly active and cozymase, thiamin and nicotinic acid somewhat less active in reducing the lag in the growth of the plague bacillus, but their ready availability in the environment will greatly facilitate the rapidity of growth and of invasion of the organism; (3) glucose and lactic acid have been found to be the best and cheapest carbon sources and serine, alanine, proline, cystine, glutamic acid, glycine, phenylalanine, tyrosine and methionine are the amino-acids which can serve as the nitrogen sources for addition to the media; (4) virulent and avirulent strains show little difference in nutritional requirements.

On the basis of the above nutritional requirements Rao (1939) prepared gelatin hydrolysate medium but it was not protein-free. Seal and Mookerjee (1940-41 and 1950) succeeded in preparing a protein-free casein hydrolysate medium, originally of Mueller (1939), and enriched it with minerals and liver extract which yielded

excellent growth of *P. pestis*. The introduction of this medium not only greatly facilitated the isolation of specific soluble protein of plague bacillus and allied organisms (Seal, 1940-41 and 1951) but also yielded a new less toxic and more immunogenic plague vaccine (Sokhey, 1942-43) The Haffkine Institute of Bombay has adopted this medium for the production of this new plague vaccine and Sokhey (1944) utilised it in the preparation of a new cholera vaccine which has been found superior to the ordinary agar-grown vaccine in the mouse protection test. The solid medium prepared by Seal (1951) by adding 2 5 per cent agar to this standardised casein hydrolysate broth was found to be almost as good as the rabbit blood agar medium, which though fully nutritive for the plague organism cannot be utilised for vaccine preparation, as it contains heterologous blood This difficulty has been completely overcome for the first time by the introduction of solid casein hydrolysate agar

(b) *Morphology and colony characters*. Bhatnagar (1940*a*, *b*) studied the morphology, growth and dissociation of plague and pseudotuberculosis organisms and classified the plague strains into virulent, avirulent protective and avirulent non-protective strains He could not support the view propounded by Sokhey (1940) that the plague bacillus possesses a capsule With the help of the Indian ink method he found that the most important structure from the point of view of immunisation was the 'envelope' substance present in the virulent and avirulent protective strains and not in the avirulent non-protective strains This substance developed best when the organism was cultivated at 37°C in 10 per cent horse serum agar containing a carbohydrate and was lost by serial sub-culture on rabbit blood agar every 48 hours at 37°C. The protection power of a live avirulent plague strain like the Java strains, Tjiwidij smooth, and the Madagascar strain-E.V., depended upon this envelope substance, but Sokhey (1942-43), by careful planning of the experiment, showed that the immunogenic power of the organism diminished as its virulence was reduced, but eventually a point was reached where it ceased to have either invasive power or protective power The protective power of the avirulent strains, according to him, was due to the residual virulence which enables the organisms to multiply in the body.

Wats and Puduval (1940) studied the morphology and colony forms of the virulent and avirulent plague strains and their dissociant forms They generally showed two types of colonies designated as 'smooth' from the virulent strain and large sized colonies with pin-pricked and sometimes rugose surface and irregular, broken or serrated margin from the avirulent strain The smooth colonies were composed of uniform oblong bacilli while the rough colonies of long forms of varying size. The former gave uniformly turbid growth in broth and the latter a granular deposit at the bottom. Seal (1951*a*) also studied a large number of strains belonging to 3 types: (1) virulent, (2) avirulent protection,

and (3) avirulent non-protection, in greater details. He also included in the study several strains of *P pseudotuberculosis* with a view to finding out whether there was any relationship between the cultural, morphological and biochemical characters of *P. pestis* and the virulence and immunizing properties of the different types. He summarised the characters as follows :

The virulent plague organisms produce colonies on rabbit blood agar the majority of which are translucent, smooth, convex and slightly viscous with or without transparent fringes and tendency to haemodigestion, producing uniform turbidity in nutrient broth with heavier growth on the top surface, forming homogeneous and fairly stable suspension in normal saline and consisting generally of short ovoid pale staining organism The avirulent protective organisms are also pale staining, ovoid or longish type, producing mostly flat, smooth and translucent colonies with or without transparent fringe, form uniform turbidity in nutrient broth and fairly stable emulsion in normal saline The avirulent non-protective strains, on the other hand, are of longish and thready forms staining more deeply, producing colonies, majority of which are rough, comparatively more opaque, with or without small transparent fringe and form granular turbidity in broth with deposit at the bottom and unstable emulsion in normal saline The pseudo-tuberculosis strains, unlike the plague strains, are motile An improvement in the motility test was effected by subculture into a semi-solid nutrient agar incubated at 22-25°C

(c) *Measurement of virulence* Sokhey (1939*d*) developed a biological method for testing the virulence of plague organism in the laboratory in-bred mice A fully virulent organism is one of which only 6–12 organism kill approximately 100 per cent mice within 3 to 11 days The relative virulence of different strains could be further particularised by the average number of days elapsing between infection and death

(d) *Maintenance of virulence* · Sokhey's (1939*a*) method of maintaining the virulence of plague bacillus consists in planting the strains to be preserved on 5 per cent rabbit blood agar slopes in test tubes, incubating then at room temperature (26°C–32°C) for 4 days and at the end of this period sealing the tube in the flame and storing them at 4° ± 2°C. For maintaining the virulence for a prolonged period Seal and Habbu (1940-41) developed a substratum in which when the organism is suspended and dried in a frozen state by cryogen process and vacuum-sealed, the organism remains viable for many years and can be regenerated at will by addition of the required quantity of distilled water. A survival rate of as high as 50 per cent has been attained and no change in virulence, serology or biochemical behaviour of the organism has been noted when regenerated.

2. Study of the chemical antegenic structure

Wats and Puduval (1940) connected the antigenic value of plague vaccine with the soluble 'envelope' substance of the plague bacillus (S S.) grown at 37°C, and found that the presence or absence of the S S did not depend upon the virulence or non-virulence nor upon the smooth or rough colony appearance but generally the rough colonies gave a poor yield.

Following a preliminary attempt by Shrivastava (1939) Seal (1940-41,1951) developed a method for isolating the specific soluble protein from the filtrates of the plague vaccine grown in casein hydrolysate broth as well as from the water soluble extracts of the plague and allied organisms grown in casein hydrolysate agar. Generally two fractions, namely, one obtained at one-third and another at (1/2-1/3) saturation of sodium sulphate were isolated from nearly all strains grown on casein hydrolysate broth but no water extractable protein fraction could be isolated from the avirulent non-protective plague or pseudo-tuberculosis strains. Their chemical, physical, serological, immunological and mouse-protective properties have been studied. Both serologically and immunologically P1/3 fraction (Antigen A,) of the virulent and relatively avirulent protective strains was found responsible for the specificity and mouse-protection whereas their P(1/2-1/3) fraction (Antigen B) was non-protective and formed the linking antigen between the virulent or protective strains and avirulent non-protective plague or pseudo-tuberculosis strains. The results thus obtained confirmed Sokhey's observation that the mouse-protective qualities of the Haffkine plague vaccine lies in its supernatant.

In this connection a polysaccharide yielding osazone resembling that of Arabinose (M P —166—168°C) was isolated by Seal (1951) from the specific soluble protein as well as from the bacterial debris of protective plague strains only and not from the non-protective plague or pseudo-tuberculosis strains Thus he concluded that the specific protective substance of plague bacillus was a polysaccharide-protein complex

On the basis of the above studies Seal (1951) classified the plague strains into the following three groups, viz , (1) those containing both antigens, A + B, e g , all virulent and relatively avirulent protective plague strains, (2) those containing only a small residual antigen A and full antigen B, e g, some strains in the process of dissociation, and (3) those which contained only antigen B, e.g., avirulent non-protective plague strains and pseudo-tuberculosis group of organisms.

3. Serological studies

Bhatnagar (1940*b*) confirmed Schutze's (1939) work about the envelope antigen but the latter was found to interfere with the somatic agglutination. This

envelope substance is absent in the avirulent non-protective plague and pseudotuberculosis strains and the plague strains are linked with the pseudo-tuberculosis strains through the common somatic antigen, but the somatic agglutination was difficult to interpret due to the salt and serum sensitivity even in such low concentration of salt as 0.1 per cent Human cases of plague in the state of recovery show both envelope and somatic agglutinins although of low titre. Bhatnagar worked out the antigenic constitution of *P. pseudotuberculosis* organisms as follows: (1) a somatic antigen shared by all the strains of this organism as well as by *P. pestis*; (2) a group specific somatic antigen present only in certain strains; (3) a type specific somatic antigen characterising individual strains, and (4) a common flagellar antigen among the pseudotuberculosis group.

Wats, Wagle and Puduval (1940) encountered the same difficulty in testing the agglutination reaction as Bhatnagar and although they could not detect serological reactions between the organisms grown at 37°C and those grown at 27°C, the latter was incapable of absorbing the antibody raised in rabbits by injecting the culture grown at 37°C The latter when heated at 100°C also behaved in the same way.

Wagle (1938) attempted to study the precipitin titres of anti-plague sera by means of antigen prepared by heating agar-free saline suspension of the plague organism at 100°C for 1 hour The titres were low and did not always correspond to the protective qualities of the sera. Menzes (1939) showed that plague infection in dead rodents could be diagnosed serologically by putting up an emulsion of spleen or liver tissue against an appropriate serum (i.e., raised against *P. pestis* grown at 37°C, titre not less than 1 300), the resulting reaction being made manifest by a heavy deposit and the titre consisting of agglutinated plague bacilli.

Seal (1940-41, 1951*b*) overcame the difficulty He obtained a stable suspension of plague strains by using an 18-20 hours rabbit blood agar culture at 37°C and preparing the emulsion slowly by means of a suspension which remained stable even when preserved in the refrigerator for a day or two. The agglutinations were found to be of two kinds, viz, floccular and granular. The former was related to surface or protective antigen was of low titre and the latter somatic antigen and normally gave high titre agglutination. Thus, the anomaly regarding high and low titre agglutination was clarified. The advantage was taken of the specific protein substances of the virulent or protective plague bacillus and an anti-serum prepared against the P 1/3 fraction of the water soluble extract or of the supernatant of plague vaccine prepared in casein hydrolysate broth gave specific floccular agglutination with virulent and protective plague organisms only and the avirulent non-protective plague and all pseudotuberculosis organism could be completely eliminated This result has been supported by precipitin and cross-complement

fixation reactions carried out with the protein fractions and mouse-protection tests. Thus Seal (1951c, 1953) succeeded in establishing three groups of strains among the laboratory collections of plague strains both by chemical antigenic analysis and by serological methods developed by him Besides, the pseudotuberculosis organisms could now be serologically differentiated by agglutination precipitation and complement-fixation tests It may be mentioned in this connection that this was the first time that the precipitation and complement-fixation test could be successfully applied to plague studies and the specificity of agglutination test thoroughly established Seal's (1952) summary table of serological relationship as published by Pollitzer (1954) in his recent comprehensive book on Plague is given below .

Serological relationship between P pestis *and* P pseudotuberculosis

Sera produced with	*P pestis* virulent and avirulent protective	avirulent non-protective	*P. pseudotuberculosis*
1. Virulent *P pestis*	+	+	+
2. Virulent *P pestis* absorbed with *P. pseudotuberculosis*	+	0	0
3 *P. pestis* boiled for ½ hour	0	+	+
4. *P. pseudotuberculosis*	0	+	+
5. Water extractable protein of *P. pestis*	+	0	0
6. *P pseudotuberculosis* absorbed with *P. pestis* boiled or *P pestis*	0	0	+

4. Epidemiology of plague

(1) *Rats in relation to plague* Sokhey and Chitre (1937) designed experiments to work out the correlation between mortality rate in the human population and degree of susceptibility of the rats in the places affected. The percentage of deaths in the several series worked out, on the whole, is inversely proportional to the human mortality. Thus Nasik City with a plague mortality rate of 3.63 per mille furnished rats none of which died in the experiment, whereas Madras City with only 0 004 deaths per mille gave a rat death rate of 91.1 per cent. Similarly, in Bombay City with a human mortality of 2 0 per mille no rat showed any evidence of susceptibility The authors favour the view that different degrees of susceptibility to plague are to be found among different races of *Rattus rattus* and that in any given locality the very susceptible races tend to die out, leaving a higher proportion of rats which are naturally resistant.

Rural plague in India is due essentially to *Rattus rattus* while *R. norvegicus* is involved mainly in cities and towns. Continuous observation carried out in Bombay since 1907 showed a change in the rodent composition by 1937, *R. Rattus* and *R norvegicus* which formed 99 per cent of the rodent population in city and were highly resistant to plague were replaced by *Gunonys varius*, a very susceptible rat to the extent of 30 per cent (Sokhey 1937).

Urban and rural manifestation of plague According to Pollitzer (1954) who made a critical analysis of the recent reappearance of plague in Bombay, zootic plague may become re-established in an urban community comparatively soon after it has become extinct. Seal (1949a) while describing the epidemiology of plague with reference to the last Calcutta epidemic was of the opinion that the plague epizootic re-established itself in the city after 22 years and was not due to importation of fresh infection In this connection he (1949a, b) described one outbreak of pneumonic plague in a village near Calcutta and another at Gaya (Bihar). On the other hand, Sharif and Narasinham (1943) who recently studied the ecology of plague in the two district of Bombay State maintained "that the idea that plague is more a rural problem is fallacious". In their opinion, the big grain centres received the infection from some infected village through fleas imported with grain, and caused a dissemination of plague, mainly through grain, to other villages. Usually, the grain centres themselves did not become seriously involved in the progress of infection because, owing to past epizootics, a large proportion of these rats was plague-resistant.

Sylvatic plague Sharif and Narasinham (1943, 1945) in Bombay and George and Timothy (1941) in Nilgiris and Rao (1947) in the Hyderabad State made investigation regarding the possible existence of sylvatic plague in these areas. In an endemic area Rao found the field rats (*Tatera indica*) highly susceptible in contrast with the house rat (*R. rattus*) which showed a fair amount of resistance. Thus, although infection among wild rodents was detected in the Barsi Taluka on eight occasions, later studies elsewhere and the studies made by George and Timothy and Rao support the view that sylvatic plague which is so important a problem in many countries does not seem to obtrude itself to any extent in India.

(2) *Studies on fleas* . It is known that out of three main types of fleas prevalent in India, *X. Cheopis* is the chief vector, while *X. brasiliensis* may be important in Decca and *X. astia* is negligible as direction vector and that plague is rare in places where *X. astia* is prevalent and *X. Cheopis* is correspondingly scarce. Rao (1940) compared the flea population of the Hyderabad city, liable to attacks of plague every year, and Nalgonda town practically free from plague except for imported cases. The fleas of Hyderabad was *X. Cheopis* and those of Nalgonda were *X. astia*, the inefficiency of the latter as a vector being ascribed by him to its great susceptibility to adverse climatic conditions. One of the causes of the absence

of plague infection in wild rodents may be due to absence of efficient flea-vector among them, for instance, the predominating rat flea found by George and Timothy (1941) in the field rats in the Nilgiris were *stivalis* and *ceratophylus*, both of which are very poor as vectors of plague infection. In the study of epidemiology of plague in Calcutta, Rao (1941) found that 60.6 per cent of the fleas population in the city was composed of the *X astia* and 39 4 per cent by *X Cheopis* and that *R rattus* which found only about 13-14 per cent of the total rat population, carried more cheopis than astia, while reverse was the case with *R. norvegicus* and *Gunomys varius* Although he could not explain why plague was absent from Calcutta he came to the conclusion that the city provided a favourable ground for its spread Sharif (1940-41, 1942-43) studied the effects of temperature and humidity upon the growth of early states of the three Indian rat fleas as also their nutritional requirement Exposure of third instar larvae of cheopis for even two minutes at 45, 45 5°C at 60, 80 and 90 per cent humidities are fatal. At 38°C and relative humidities 80 to 100 per cent *cheopis* died within 3 days and brasiliensis within 30 hours but astia showed signs of growth At 100 per cent humidity they lived for 3-5 days and at 97 5 per cent humidity for 5-11 days At 13°C larvae of all species completed their development at 80 to 100 per cent humidity The resistance to cold 0° to 2°C at 80, 90 and 97.5 per cent humidities was also not considerable, larvae and pupal stages surviving up to 6 days only. The unfed larvae is more resistant and pupae more resistant than larvae. The duration and life of infected fleas is very short especially in the warm and dry months and they can only be transporated to short distance, accompanied by a high rate of mortality amongst them

In regard to the nutritional requirement it has been experimentally proved that certain accessory food substances such as vitamin B are needed and blood is not so essential for the successful breeding of larvae but better results are obtained when blood forms an integral part of food. In addition, the association of some micro-organisms with the food ensure a more successful breeding of the larvae. The author (Sharif 1944-46) suggested that irregular patchy distribution of the three common rat fleas in India and their host preference are governed by the resting conditions and nature of the food of their hosts in combination with the climatic conditions of the place The dietetic requirement of larvae also vary according to species *X astia* requires more nutritive diet than those of other two species and its temperature tolerance is also higher, while *X cheopis* can thrive with comparatively poor diet and temperature tolerance is not so high as in *astia*. *X. brasiliensis* larvae thrives better on cereal proteins than those of the other two types. There is greater multiplication of fleas and plague organisms in them during cooler months and a severer check on their activities during the hot and dry month of the summer.

According to Sharif and Narasinham (1940-41), the increase in the flea index

of the domestic rats is governed by three important factors which work independently of one another, (1) the climatic factor, (2) storage of grains in the premises increases flea population on account of abundance of food for rats and flea larvae, and (3) the existence of plague infection on the premises increases flea population as a result of plague epizootic rather than as a cause for it.

In a recent study on the ecology of fleas by Seal (1953) in connection with the newer re-appearance of plague in Calcutta an important observation has been made.

(3) *Endemic centres and spread of infection*: Sharif (1942-43, 1951) from the analysis of about 7 years epidemics in the southern Registration Districts of Bombay Province observed that plague has clung since long to cool and comparatively damp areas comprising of water shed situated in the sub-mountainous region and high table lands varying in altitudes from 2,000 to 4,000 feet. They can conveniently be designated as endemic centres, although plague moves from place to place even in these areas Three such centres are located in the Himalayan sub-mountainous region and one in the water shed of the Central India Mountain ranges and there are 3 endemic centres in the southern region Plague radiates from these endemic centres and the limits of the infected areas increase or decrease gradually

5 Cellular response in plague infection

The cellular response in plague infection was studied by Bhatnagar and Shrivastava (1946). The envelope antigen corresponding to the antigen A of Seal (1940-41 and 1951) was found responsible for the specific clasmatocytic response as well as protection, whereas the somatic antigen did neither give this specific cellular response nor afford protection to the animal against infection. The prognostic significance of the leucocytic count was studied by Wagle and Colah (1947) in bubonic plague. Excluding the probable sources of error the optimum condition for prognosis was curiously found to be the normal leucocytic count, i e., between 5,000 and 10,000 per cent. A count below 5,000 was found as bad as a high count, the former being indicative of absence of resistance and the latter depending upon the degree of septicaemia which was determined by actual colony count. Actually, a death-rate of 97 3 per cent was recorded in cases giving a count of 40,000 or more

6. Treatment of plague

(1) *Serotherapy*: Normal-Walker (1937) treated plague cases with sera of human convalescents obtained after the temperature had been normal for 10 to 15 days. Forty-eight cases treated with 20 cc of serum or near amount on three suc-

cessive days gave a mortality rate of 16 7 as against 47.9 per cent in the same number of controls

Sokhey (1938) treated 69 cases of plague with anti-plague horse serum with 27 per cent mortality as against 65 per cent among the 55 cases in the control group (treated with I V. Iodine) of the 34 bubonic cases there was only one death, 4 out of 15 died among the mild septicaemic cases and 14 out of 20 cases died amongst the severely septicaemic cases In a recent summary by Sokhey, Wagle and Habbu (1953) the fatality rate of 157 cases treated by anti-plague serum was 23.5 per cent whereas in 71 bacteraemic cases it was 50 7 per cent Clinically it was noticed that the administration of the serum markedly reduced the toxic symptoms within 24 hours, delirium, restlessness and congestion of the eyes disappeared and temperature fell Experimentally, Seal (1951, 1953) found anti-plague serum raised in rabbits, gave the best protection against plague infection

(2) *Chemotherapy*

(i) *Sulpha drugs* Of the 35 compounds synthesized by Nandi and Ganapathi (1940) six were tested against plague infection in mice by Sokhey and Dikshit (1940) and remarkable curative action was noted with sulphathiazole, which was much superior to sulphapyridine (MB 693), the only other drug then tested against plague infection In septicaemic cases the cure rate with sulphathiazole was 80 to 90 per cent as against only 10 per cent in sulphapyridine treated group.

Following the above results, Wagle, Sokhey, Dikshit and Ganapathi (1941) carried out well controlled trials in 237 bacteriologically verified plague cases by the following 4 methods of treatment, namely, (1) anti-plague serum, (2) sulphapyridine (MB 693), (3) sulphathiazole and (4) iodine solution intravenously. Taken in the above order the number of cases treated were 70, 53, 32, and 82 with case mortalities of 28.5, 24 5, 15 6 and 52 4 per cent respectively. If only septicaemic cases are considered the serum case mortality worked out at 60.6 per cent as compared with 43 8 and 41 8 per cent respectively of sulphapyridine and sulphathiazole and iodine control showed a mortality of 95 per cent.

Sokhey and Wagle (1946) followed up the therapeutic trial with another drug *sulphadiazine* and compared the results with other sulpha drugs and iodine treatment. Of the 1,604 septicaemic cases treated in the fields with anti-plague serum, *sulphapyridine*, *sulphathiazole*, *sulphadiazine* and *iodine* intravenously the mortality rates were 50 7, 50 0, 41 6, 32 0, 20 9 and 91.0 per cent respectively. Thus sulphadiazine showed significant reduction in mortality. Sulphathiazole doses were — initial 2 g, 2 g 4 h later and then 1.5 g every 4 h till 24 h — maximum period — 10 days, sulphadiazine doses, initial 4 g followed by 2 g every 4 h — period of treatment not more than 10 days, some alkaline mixture should be prescribed and plenty of water to drink. These findings are sup-

ported by the work of Simeons and Chhatre (1946) who treated 1,000 cases of bubonic plague with either sulphadiazine or sulphathiazole These workers (Simeons and Chhatre 1947) treated another series of 700 cases with *sulphamerazine* the mortality rates being 14 per cent for septicaemic cases and 7 2 per cent for bubonic cases as against 18 per cent and 8 per cent respectively with sulphadiazine Although there was practically no difference between the two drugs sulphamerazine was preferred by the authors as the interval between the doses given I V. could be prolonged to 8 h instead of 4 h in case of sulphadiazine.

Sokhey and Habbu (1947-48) in their extended work with sulphonamides in experimental plague infection in mice included two antibodies, namely, penicillin and streptomycin These antibiotics were given subcutaneously, the former in doses of 1,000 units 4 times a day for 7 days and the latter in doses of 0 8 mg 4 times a day for the first 4 days and 0.2 mg four times a day for the next 5 days, the other sulphonamide drugs tried being *sulphadiazine*, *sulphamerazine*, *sulphamethazine* in their usual doses for mice No protection was given by penicillin, whereas no deaths were recorded in a group of 10 mice treated 48 h after infection with streptomycin and only 1 death out of ten when treated 72 h after infection The corresponding death rates with sulphamerazine, sulphamethazine and sulphadiazine is being 30, 30 and 50 per cent respectively. Thus, the action of streptomycin was definitely superior to those of sulphonamides. Wagle and Sokhey (1948) followed this up in human cases, bacteriologically confirmed 332 cases were treated with one of the three drugs, streptomycin, sulphamerazine and sulphadiazine separately Considering all cases the mortality rates with the three drugs were 4 2, 7.2 and 7.0 per cent respectively The same for the septicaemic cases only (at the commencement of treatment) were 10.8, 31.8 and 21.0 per cent respectively.

Datta Gupta (1948) compared the effect of sulphamezathine with that of sulphadiazine in human cases and obtained practically the same result, while Patel and Rebellow (1948) noted marked improvement in the result of sulphonamide treatment in bubonic plague cases with history of previous plague vaccinations.

Since the efficacy of plague treatment with sulphonamides depends upon the early treatment before septicaemia starts and upon the maintenance of a high level of the drugs in the blood the WHO expert committee who met in Bombay in 1952 recommended that the early cases of bubonic plague could be effectively treated using a dose of about 10 g (2 gr I.V.) on the first day, followed by smaller doses making a total of 50 g during the first week and that the treatment should be continued for at least 3 days after the temperature becomes normal.

(ii) *Combined sero and chemotherapy:*

Sokhey and Wagle (1940-41) summarised the results of the five field trials with the various sulpha drugs and anti-plague serum during the period between 1936 and 1940 The total number of cases treated separately with anti-plague serum, sulphapyridine, sulphathiazole, sulphathiazole with anti-plague serum and iodine intravenously was respectively 157, 122, 230, 60 and 149, the average respective case mortality being 23 5, 27 0, 20 8, 20 0 and 53 6 per cent. If the results are analysed for the mild and severe septicaemic cases the corresponding mortality rates for the former group were 24 1, 19.2, 14 8, 0 0 and 73 7 per cent and the latter group 69 0, 72 2, 55 4, 38 1 and 96 4 per cent respectively Thus, in this series sulphathiazole gave the best results, which could be further improved when the treatment was supplemented by the anti-plague serum

Antibiotic treatment:

(a) *Penicillin and streptomycin* According to Gupta *et al* (1946) and Sokhey and Habbu (1949) penicillin had no effect on experimental plague infection in mice. On the other hand, streptomycin proved extremely valuable both in experimental as well as in human plague cases The rates of survival of the bubonic cases treated with this drug were 83 3 per cent by Datta Gupta (1948), and 80 per cent by the same authors (1949) in another series, 90.6 per cent by Wagle (1948), 96 1 per cent by Ghosh (1950), 96 2 per cent by Rao (1952), 95 8 per cent by Sokhey *et al* (1948) and 100 per cent by Dubey (1953) All of five primary pneumonic cases treated with streptomycin, anti-plague serum and sulphamerazine by Wagle and Bedarker (1948) survived Seal (1949*b*) also reported that two cases of primary pneumonic plague one at Calcutta and another at Gaya treated with streptomycin survived. In pneumonic cases sulphonamide and/or penicillin should also be used to prevent secondary infection or their sequels (WHO Expert Committee 1952).

(b) *Aureomycin and chloramphenicol*.

Experimental trials with aureomycin and chloramphenicol in plague infected cases have yielded favourable results in the hands of Sokhey and Habbu (1950). However, in order to obtain identical results, 42 mg of aureomycin or 336 mg of chloramphenicol had to be administered as against 10.4 mg of streptomycin. Ramchandran (1952) recently recorded the results obtained with aureomycin treatment in 12 bubonic and 3 septicaemic plague patients. Three of these including 2 septicaemic cases died

7. Standardization of Anti-Plague Horse Serum

Sokhey (1938) introduced a biological assay of anti-plague horse serum in

mice on the same lines as the plague vaccine. For testing the protective power of a serum ten times the m l d. of a virulent plague strain (60-120 organisms) is given per animal subcutaneously. For any given serum five graduated doses near 50 per cent endpoint (determined by preliminary test) are selected. Serum dose and the standard test infective dose are given at the same time subcutaneously but in different parts of the abdominal wall and the mice observed for 30 days Alternately the serum doses are given divided into four equal doses, first dose being given 72 hours after the standard infective dose (6-12 organisms of virulent *P pestis*) and the remaining portions are given subcutaneously at 24 hours interval (between 4th to 7th day). The mice are then observed for 30 days. Those which die are postmortemed and examined for plague bacillus and those which survived 50 days period were killed and examined in the same way. If *P. pestis* is not found it is noted in the protocol as plague negative and the 50 per cent end point is calculated according to the method of Reed and Muench (1938). A suitable anti-plague serum should have a minimum mouse protective dose of not more than 0 05 ml or minimum mouse curative dose of 0.4 ml

Wats (1938) obtained encouraging results in the *in vitro* titration of anti-plague serum by means of precipitin reaction with a lysate of *P pestis* culture grown on meat digest agar at 37°C and treated with chloroform or a detergent product called 'Lissopol' solution (I C I). The test was put up according to Dean and Webbs method of optimal properties against 1 100 of serum and incubated in a waterbath at 55°C The results obtained were said to be regular and repeatable and more or less similar to the biological test though the order of potency was different This work was not pursued afterwards.

Seal (1940-41, 1947) developed quantitative precipitin nitrogen, agglutinin nitrogen tests according to Heidelberger's Technique and flocculation test by means of the P1/3 fraction of the specific soluble protein of the virulent plague bacillus A statistically significant correlation has been found between the agglutinin and precipitin nitrogen values and mouse protective units, one mouse-protective unit being the amount of serum which would give 50 per cent survival of a group of mice against the standard infective dose of Sokhey (100-120 organisms). For the preparation of standardised suspension for the quantitative agglutinin nitrogen test the organism (virulent *P. pestis*) was grown in casein hydrolysate broth at 28°C for 72 hours and killed by 0.1 per cent formation The development of these *in vitro* test permits a rapid estimation of the potency of the serum particularly from animals that are under immunization to facilitate bleeding and immunization procedures and obviates the time-consuming biological tests which can be reserved for the final corroborative test before the release of the serum for therapeutic use. The flocculation test developed by him is only a rough and ready method while the agglutinin nitrogen test is easier than the precipitin nitrogen test though the latter is more accurate than the former. In this connec-

tion, Seal prefers rabbits to horse as an animal of choice for the production of therapeutic anti-plague sera

Prophylactic plague vaccine.

The laboratory of the Haffkine Institute, Bombay is the only place in India (except perhaps one or two States) where prophylactic plague vaccine is prepared and supplied to the whole of India and sometimes to outside countries During the last decade various important studies have been made and a considerable improvement in the quality of vaccine has been effected The most important contribution immediately prior to this decade was the introduction of the biological assay of the potency of vaccine by Sokhey and Maurice (1935). Since then this method has been rigidly followed in assessing the potencies of various types of vaccines prepared from time to time for experimental purposes in that Laboratory.

The optimal temperature of growth, period of incubation, *p*H of the media, etc. have been statistically standardised The optimal temperature of broth vaccine is 28°C whereas the same for agar-grown vaccine is 37°C. The claim of Girard and Robic of Madagascar and of Otten in Java that the live avirulent immunogenic vaccine has definitely greater protective power than the killed vaccine has been confirmed by Sokhey and Habbu (1940-41, 1942-43), who also showed that it was possible at all to reduce the virulence of an organism and still retain a high degree of immunogenicity but then the strain to have immunogenicity must also have some virulence (residual virulence) and again once the organisms are killed whether virulent or relatively avirulent the protective power of the vaccine falls to about the same level The higher protective power of the avirulent live vaccine was according to Sokhey due to the retained residual virulence to enable it to multiply in the body as shown experimentally in mice. He could not, however, support the idea of using live vaccine due to various other objections.

In testing the protective power Sokhey and Habbu (1942-43) did not find any difference between rat and mouse.

The best prophylactic plague is now being prepared in casein hydrolysate broth introduced by Seal and Mukherji (1940-41). The vaccine prepared in these media is highly protective (the mouse protective dose approximately that of agar-grown vaccine, e.g., 0 004 m), easy to prepare, possess high keeping qualities, very much less toxic, particularly when the organisms are killed by 0 075 per cent formalin and preserved in 1 mg phenylmercuric nitrate per 100 cc the toxic dose being o c. ml compared to 0 2 ml of the original Haffkine broth vaccine (Sokhey and Habbu, 1944-46). This vaccine is now prepared from three days' growth instead of 4 weeks' growth at 28°C, thus the period necessary to prepare the vaccine has

mice on the same lines as the plague vaccine. For testing the protective power of a serum ten times the m l.d. of a virulent plague strain (60-120 organisms) is given per animal subcutaneously. For any given serum five graduated doses near 50 per cent endpoint (determined by preliminary test) are selected. Serum dose and the standard test infective dose are given at the same time subcutaneously but in different parts of the abdominal wall and the mice observed for 30 days Alternately the serum doses are given divided into four equal doses, first dose being given 72 hours after the standard infective dose (6-12 organisms of virulent *P pestis*) and the remaining portions are given subcutaneously at 24 hours interval (between 4th to 7th day) The mice are then observed for 30 days. Those which die are postmortemed and examined for plague bacillus and those which survived 50 days period were killed and examined in the same way. If *P. pestis* is not found it is noted in the protocol as plague negative and the 50 per cent end point is calculated according to the method of Reed and Muench (1938). A suitable anti-plague serum should have a minimum mouse protective dose of not more than 0 05 ml or minimum mouse curative dose of 0 4 ml.

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The optimal temperature of growth, period of incubation, *p*H of the media, etc. have been statistically standardised The optimal temperature of broth vaccine is 28°C whereas the same for agar-grown vaccine is 37°C. The claim of Girard and Robic of Madagascar and of Otten in Java that the live avirulent immunogenic vaccine has definitely greater protective power than the killed vaccine has been confirmed by Sokhey and Habbu (1940-41, 1942-43), who also showed that it was possible at all to reduce the virulence of an organism and still retain a high degree of immunogenicity but then the strain to have immunogenicity must also have some virulence (residual virulence) and again once the organisms are killed whether virulent or relatively avirulent the protective power of the vaccine falls to about the same level The higher protective power of the avirulent live vaccine was according to Sokhey due to the retained residual virulence to enable it to multiply in the body as shown experimentally in mice He could not, however, support the idea of using live vaccine due to various other objections

In testing the protective power Sokhey and Habbu (1942-43) did not find any difference between rat and mouse

The best prophylactic plague is now being prepared in casein hydrolysate broth introduced by Seal and Mukherji (1940-41) The vaccine prepared in these media is highly protective (the mouse protective dose approximately that of agar-grown vaccine, e.g, 0.004 m), easy to prepare, possess high keeping qualities, very much less toxic, particularly when the organisms are killed by 0.075 per cent formalin and preserved in 1 mg phenylmercuric nitrate per 100 cc the toxic dose being o c. ml compared to 0.2 ml of the original Haffkine broth vaccine (Sokhey and Habbu, 1944-46). This vaccine is now prepared from three days' growth instead of 4 weeks' growth at 28°C, thus the period necessary to prepare the vaccine has

been considerably reduced to that of agar-grown vaccine with the minimum of manipulations.

Control of plague:

The advantage of early preventive measures including prophylactic vaccine inoculation were stressed by James (1939) and Bhargava (1939) The incidence of plague cases among those receiving one dose of Haffkine plague vaccine was found by Patel and Rebello (1948) to be 12 3 per cent as against 32 5 per cent among the unvaccinated group exposed to the same risk.

Raticidal and pulicidal measures:

Ahluwalia (1940) studied the role of calcid fumigation as a raticidal and pulicidal measure in anti-plague campaign and concluded that calcid was superior to cyanogas both of which were better than carbon monoxide and sulphur dioxide gas fumigants HCN content of calcid is twice that in cyanogas and the briquettes are easy to keep but cyanogas is less dangerous. These findings found support in the work of Sokhey, Chitre and Gokhale (1940) carried out on the same line. Sharif (1944-46) reported favourably on the efficiency of 'Antu' as a rat-killer of *Rattus norvegicus* type but not of *Rattus rufescens* and *Gunomys kok.*

D D T and gammexane·

Dusting rat artificial rat burrows with 10 per cent D D T with foot pump killed fleas even after 998 days up to 9½ feet. The effect is reduced to 75 per cent killed at a distance of 11¾ feet and to 33 per cent at a distance of 19½ feet up to a period of about 2¾ years. Experience with aqueous solution showed that 0.297 mg of D D.T. per square cm continued killing fleas 100 per cent fleas up to 579 days tried (Sharif 1947-48).

The same author (1947-48) used gammexane smoke and 3 per cent gammexane (I C I.), and obtained 100 per cent kill of rat fleas tried after 45 days of the smoking the burrow at a distance of 20'4" from the entrance. This smoke was not poisonous to the domestic rats when kept in the burrow for 24 h.

Khatri (1953) reported a remarkable reduction in the incidence of plague in the Hyderabad State following a systematic campaign of flea destruction by D.D T. Prior to the adoption of the method in 1949, 6,858 cases with 2,069 deaths were reported; after 3 years of regular spraying with D.D.T. this figure came down to 17 cases with 2 deaths in 1952

Wagle and Seal (1953) in a report submitted in 1952 to the WHO Expert Committee on Plague compared the results obtained in various parts of India from 1945 with D D.T., B.H C. and Cyanogas in the control of plague. The results of

the Cyanogas fumigation shows that used alone it does not prevent the annual recrudescence of epidemics but this can be achieved by the application of D.D.T. despite certain failures in a few instances In one series of experiments the toxic action of both D D T. and B H C (Gammexane) was still evident 84-90 days after dusting but the D.D T. was the more powerful palicide. Cyanogas dusting had no effect in reducing the flea index in these experiments In their conclusion the authors consider that D.D T is the most valuable pulicide, followed by B H.C. and Cyanogas, in that order A successful anti-plague operation, according to the authors, however, depends on the methods used, the dosage of pulicide, and the time and intensity of its application In addition, to eradicate plague in endemic areas, it is also necessary to continue control efforts during the inter-epidemic periods

The methods of D D T application mainly utilised in India for the purposes *of plague control were (a) indoor residual spraying with emulsions containing* 5 per cent of the insecticide laying a deposit of 70-75 mg of fresh D D.T. per sq *ft.* and/or (b) *treatment (insufflatu) of the rat burrows with 10 per cent D.D.T.* dust at least during inter-epidemic period as advocated by Viswanathan and Rao (1947, 1949). It is of great importance to note that the application of D D T was found not only far more effective but also more economical than that of Calcium cyanide. In an operation covering 90 villages the cost was found to be Rs 1,750 as against the probable cost of Rs 4,500 if Cyanogas was used and that also with *no good result.*

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During this period of thirteen years the work on leprosy has made much headway in all directions. The work can be divided into the following sections:

(1) Clinical

(2) Chemical and biochemical

(3) Therapeutic

(4) Bacteriological

(5) Immunological

(6) Histological

(7) Animal experiments

(8) Epidemiological

Clinical

Lowe and Chatterji (1938) reported about extensive ulceration of the skin in leprosy. This was an unusual phenomenon and found in two cases of tuberculoid leprosy in the phase of reaction The chief difference between lazarine leprosy and the two cases recorded was the absence of bacilli or scanty bacilli in the lesions Gupta (1938) reported about a case of Pellagra simulating leprosy Lowe (1938) made a study on racial variations in leprosy with particular reference to Indian and Burmese races Das (1938) narrated a case of accidental transmission of leprosy. Soon after that Lowe and Chatterji (1939*a*) published an article on "Scarification, Tattooing, etc. in relation to leprous lesions of the skin" They recorded the occasional appearance of leprous lesion at the site of a previous scar might occasionally be transmitted by infected instruments used in scarification, tattooing or vaccination They considered it more common for the appearance of a leprous infection which was latent at the time of injury They also noticed that leprous lesions were often treated by scarification, application of cautery or of caustics, and by tattooing. These procedures caused marked scarring and made subsequent diagnosis difficult Rishi (1939) reviewed arrested cases and observed that 10 8 per cent cases relapsed Lowe and Chatterji (1939*b*) observed seasonal variations in leprosy in Calcutta They reported larger number of attendance of new cases between March and October than in the rest of the year Seasonal variation was found in neuro-macular and not in lepromatous cases without macules The clinical sign of activity was in the form of thickening and erythema and radial extension in the lesions During the month of March, April and May the percentage of neuro-macular lesions in which bacilli were found in smears rose markedly. The seasonal variation in Calcutta appeared to be related to meteorological conditions, the period of increased bacterial

activity being confined to the hot, relatively dry season and ending abruptly with the on set of rainy season. Dharmendra and Chatterji (1940) made a study of the dermal leishmaniasis cases referred to the Leprosy Department of the School of Tropical Medicine, Calcutta, on suspicion of leprosy. They observed close similarity between lesions of dermal leishmaniasis and certain lesions of leprosy and also pointed out the differential diagnosis between the two diseases The possibility of the co-existence of both the diseases in the same patient was also pointed out. Lowe and Chatterji (1940) reported the on set of leprosy with localised lesions rapidly becoming lepromatous. Later these localised patches were recognised as localised lepromatous lesions and the generalised lesions appeared within a few years. Dharmendra and Mukherjee (1943) confirmed the findings of Lowe and Chatterji about the seasonal variations in the activity of lesions of neuro-macular cases of leprosy. Prabhu (1946) made an exhaustive study of the leprous lesions in the upper respiratory passages. Chatterji and Dharmendra (1947) studied lagophthalmos in leprosy and described its mechanism, prevention and treatment

Chemical and Biochemical

Before the advent of sulphone drugs hydnocarpus oil was the sheet anchor in the treatment of leprosy But while some samples of oil caused very little pain, other samples caused considerable pain leading to considerable local irritation and sometimes abscess formation That was the experience of workers all over the world De (1938) investigated this matter and found out that the irritant products in the oil were the oxidation products of the oil, and the simplest way to test the suitability of the oil for injection was to test for the presence of free fatty acids and peroxides in the oil and diminution in the special rotation. He also suggested measures for proper extraction and storage of the oil. Basu and Mazumdar (1939) studied the keeping properties of hydnocarpus oil and found that creosoted oil kept much better than the natural oil. Therefore the oil should not be stored uncreosoted Dharmendra and De (1939) worked on blood cholesterol in cases of leprosy and noted that the administration of hydnocarpus oil was attended with an increase in the blood cholesterol in those cases which showed some improvement after treatment. Venkatasubramanium (1941) made an enquiry into the biochemical changes in the blood in leprosy. The average values for both calcium and phosphorus were within normal limits. Phosphatase activity was within normal limits except in 4 cases where there was an increase due to bony changes. There was slight reduction in total protein which might be due to malnutrition

Therapeutic

Gass (1938) injected cobra venom for the relief of leprous neuritis and reported very favourable results But one or two other workers in India using similar

methods obtained less favourable results Bose (1938) confirmed the beneficial effects of infiltration of hydnocarpus oil around the trophic ulcer and by the side of the thickened posterior tibial nerve, as reported by Lowe and Chatterji (1938). Later Cochrane (1940) confirmed the same findings and stated that all ulcers do not respond to injection treatment, and indiscriminate injections of ulcers will only bring an excellent method into disrepute Mehta (1939) obtained good results by giving similar injections containing Rivanol, glucose, calcium lactate, sodium thiosulphate and water Das (1940) did not confirm this Khan (1939) advocated rest by putting the affected foot in plaster of Paris Lowe and Chatterji (1939*c*) supported the earlier findings of Chatterji that the decapsulation of the ulnar nerve is beneficial in suitable cases Chatterji made further studies and in the All-India Leprosy Conference held at Madras in 1950 he discussed in detail about the efficacy of decapsulation, injection, of hydnocarpus oil in the affected hands, massage and exercise etc in the prevention and the treatment of deformities due to involvement of the ulnar nerve Dharmendra and Chatterji (1939) reported the results of total excision of early nuero-macular lesions In suitable cases the complete excision of a lesion is not likely to be followed by a local recurrence of symptoms, at least in a certain percentage of cases. The operation is free from any harm Chatterji (1941) described a conservative method of removing the terminal phalanges in leprosy patients without giving rise to any deformity. Chatterji and Dharmendra (1947) advocated injections of hydnocarpus oil in the affected eyelids and adjoining skin for the prevention and correction of lagophthalmos. The results were illustrated by photos taken before and after treatment. Gass (1947) reported beneficial results of massage in leprosy. Dharmendra and Chatterji (1948) studied the results of sulphone drugs (Promine, Diasone, and Sulphetrone) in 50 cases of the lepromatous type The results were encouraging Cochrane had similar experience but he was of the opinion that these drugs should be handled with care Desai noticed severe iritis in a case after prolonged treatment with Bromine and that leads to loss of eyesight. In the All-India Leprosy Workers' Conference held in Calcutta in 1948 both Cochrane (1949) and Dharmendra (1949) gave an account of their further experiences with sulphone drugs Cochrane was of the opinion that sulphones should be given to lepromatous cases and not to tuberculoid and neuro-anaesthetic cases for fear of exacerbation of the disease He was not in favour of giving smaller doses. In order to minimise the cost of treatment he suggested injections of 50 per cent Sulphetrone. Dharmendra was more enthusiastic about the use of sulphones and advocated their use in as many cases as possible. Chatterji (1949) reported the efficacy of smaller doses of Diasone and Sulphetrone by injections The reports of the conference were published in Leprosy in India in April, 1949. Further investigations on the therapeutic value of the sulphone drugs (Diasone and Sulphetrone) in leprosy were continued in 1949 and 1950 The work was summarised by the Editor (1950) in *Leprosy in India*, in April, 1950. There was a limited scope for using sulphone

drugs at that time on account of the high cost of treatment. Efforts were made to minimise the cost in two directions, firstly, the parenteral administration of the sulphone drugs commonly used by mouth, and secondly, the use of the parent drug, diamino-diphenyl-sulphone, either by mouth or parenterally. Both Cochrane and Dharmendra considered the parenteral administration of sulphetrone more economical than but as efficacious as the oral administration Dharmendra preferred the injections of 50 per cent sulphetrone, 4 c c twice weekly. Later Dharmendra and Chatterji (1950) experimented with diamino-diphenyl-sulphone in leprosy cases It was a very extensive and elaborate work By this work the suitable dose and the best method of administration of D D S, which is a very toxic drug, was found and therefore its mass use became possible It was found out that a dose of 50 mg to 200 mg daily for 6 days in the week, was quite safe for out-door treatment.

Bacteriological

From time to time attempts were made to cultivate the *Mycobacterium leprae* in different media without success Dharmendra and Lowe (1938) attempted to cultivate *M. lepraemurium* in special gaseous environment as recommended by Soule and Mckinley but there was no conclusive evidence of multiplication Similar were the results in ordinary tissue cultures, in cultures of chick leucocytes, rat bone-marrow, in minced chick embryo, and on split protein medium as recommended by Duval. Attempts to culture the organism of leprosy from the blood by the method of Lowenstein gave negative results. Chatterji (1943) reported the presence of leprosy bacillus in the thickened nerves in neural cases where the routine smears from skin lesions were negative Smears from the nerve sheath were positive in 17 per cent of the cases, in nerve fibres in 52 per cent cases, and in nerve abscesses in 94 per cent cases Dharmendra and Sen (1946) examined the nasal smears of 1,300 new cases from which skin lesion smears were negative. Bacilli were found in the nose in two cases It showed the importance of examination of the nasal smear as a routine before declaring a case as "open" or "closed" and also for the purpose of preventive measures Row (1946) attempted culture of leprosy bacillus in symbiosis with leishmania and claimed success but it was not confirmed by other workers.

Immunological

Lepromin test was originated by Mitsuda and developed by Hayashi and also investigated by Muir and Cochrane in India, as a result of which certain opinions were formed regarding the value and nature of the test. Later workers in other countries, particularly Rotberg, advanced views about the nature of the test which differed rather markedly from the views previously held. The truth of those views had to be proved by experiments and observation.

A series of studies of the lepromin test was carried out in the Leprosy Department of the School of Tropical Medicine, Calcutta, and reported in a series of articles The first article of this series was by Lowe and Dharmendra (1940). It dealt with the standardisation of lepromin for use in the classical lepromin test The second and third articles *by Dharmendra and Jaikaria (1941) and Dharmendra (1941a)* indicated that the classical lepromin test of Mitsuda may be replaced by another test. The new reaction could be read in 24 hours instead of three weeks and it was very similar to allergic reactions in other diseases Lowe and Dharmendra (1941) *reported early reaction to lepromin and a high degree of agreement between the* early and the late reactions was recorded They confirmed the report of Fernandez that the early reaction is caused by soluble antigen Early reaction was accentuated by injecting a suspension of ground lepra bacilli and the late reaction was diminished Positive results were obtained not only in leprosy cases and their contacts but also in non-contacts Dharmendra (1941*b*) extracted an antigen from leprosy bacilli which was protein in nature and soluble in saline It gave early reaction but no late reaction Dharmendra and Lowe (1942) observed that the Mitsuda Test has some value in prognosis in cases of leprosy of all types Dharmendra, Lowe and Mukherjee (1942*a*) observed variations in the result of the Mitsuda Test in neuro-macular cases and considered that there were at least five possible factors which, acting singly or in combination, might influence the results of the test These factors were variations in the clinical activity, variations in bacteriological activity, variations in the time of the year at which the test was done, deterioration of the lepromin on keeping, and the use of different lots of lepromin Of these no 1 and no 3 were the two most important factors Dharmendra, Lowe and Mukherjee (1942*b*) failed to enhance the reaction to lepromin by repeated testing in the majority of the neural cases and all lepromatous cases in which the original result was negative or weakly positive. Dharmendra (1942) devised a method of standardising lepromin from dried and partly defatted leprosy bacilli This is known as the chloroform method of extraction of bacilli, which yielded three times more *bacilli than the ordinary method It produced both early and late reaction in neural* cases and no reaction in lepromatous cases The refined material helped accurate standardisation and it had better keeping properties Cochrane, Rajagopalan, Santra and Paul Raj (1941) studied the lepromin reaction with special reference to contacts.

Histological

A long-term study of the selected cases of leprosy was made These cases had a typical feature in the lesions. The purpose of the study was to correlate the clinical, bacteriological, histological, and immunological findings with the prognosis of the disease in these cases. In this study were included the following types of lesions: (a) lesions of doubtful classification, (b) localised skin lesions of

the lepromatous type, (c) flat patches of the neural type with ill-defined margins, (d) tuberculoid patches of the neural type with ill-defined margins and smooth and shiny surface (e) tuberculoid lesions in the phase of reaction, (f) lesions of all types in children, and (g) lesions suggestive of leprosy but with no definite signs

The following were the findings .

(a) Cases of doubtful classifications. In a small number of leprosy cases the clinical findings were not clear-cut, the lesions being typical neither of the neural nor of the lepromatous type, but having some features of both the types. In some of these cases histological examination cleared up the classification, but often atypical clinical findings were associated with atypical histology Histologically the lesions were a typical tuberculoid in some, lepromatous in others and doubtful in a few others

(b) Atypical lepromatous cases These were cases of lepromatous type in whom some or all of the lesions were localisd and bacteriologically strongly positive. Histologically the lesions were lepromatous with a tendency to tuberculoid arrangement of granuloma cells.

(c) Flat patches of the neural type with ill-defined margins *Histologically* the lesions were either simple with non-specific granuloma or mild tuberculoid.

(d) Tuberculoid lesions in the stage of reaction Histologically all the lesions were tuberculoid though not typical; the atypical features being a few or no giant cells, or marked vacuolation of some cells, and of little infiltrated nerves in the midst of granuloma

(e) Cases of leprosy among children· It is generally believed that the prognosis of leprosy in children is bad. This study showed that in children having tuberculoid lesions the prognosis is as good as amongst adults having similar lesions.

(f) Cases with suggestive but no definite signs of leprosy: Some of these cases later developed definite signs of leprosy but in others the indefinite signs disappeared or turned out to be some other skin disease.

Animal Experiments

Attempts were made to transfer human leprosy to splenectomised monkeys, but failed. Sulphanilamide and M & B 693 were found to have bactericidal action on the bacillus of rat leprosy *in vitro* but the results *in vivo* were not encouraging

Epidemiological

Lowe, Dharmendra and Sen (1941) made epidemiological clinical studies in the Bankura District of Bengal and reported the findings in *Leprosy in India,*

October, 1941. Verghese and Rath (1942) made similar studies in Orissa Epidemiological leprosy surveys were made by Santra in Bihar, Central Provinces, Madras, Berar, Bombay and other places. Joseph (1939) studied the factors influencing the incidence of leprosy in the Madras Presidency. Lowe and Santra (1940) made epidemiological studies in Santalpur (north Bengal). Cochrane and Rajagopalan (1943) made a study of family susceptibility in relation to the epidemiology of leprosy They concluded that it could not be categorically stated that the family susceptibility had no influence on the development of the more serious signs of leprosy but it might be a minor factor in the epidemiology of the disease.

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DIPHTHERIA AND CLOSTRIDIA

Studies on diphtheria in India involved various aspects of the problem on its microbiology, immunology and epidemiology Pasricha and his associates (1932) made Schick test on 241 Indians and Anglo-Indians. Higher percentage of non-reactors was found amongst the Indian. Pasricha and Panja (1940) reported 5 cases of cutaneous uclers caused by mitis from of *C diphtherae* Pandalai (1945) with an idea to investigate into the carrier rate of *C diphtherae* in Vizagapatam cultured throat-swabs from 707 children between 2 to 12 years of age over a period of nearly three years *C diphtherae* was isolated from six of these children. Four of such 6 strains were pathogenic and 2 non-pathogenic Soman and Nail (1949) from a culture study of 500 throat-swabs isolated 175 strains of *C diphtherae* of which 167 were *mitis* type, one *intermedius* and 7 *gravis* Lefflers medium was found slightly inferior to Neill's blood tellurite agar

Ghosh and Roy (1939) in their investigation on the kinetics of antigen-antibody-reaction have observed that Danysz phenomenon with diphtheria toxin and antitoxin becomes apparent between the *p*H range of 4.2 and 9.6 Seal and Johnson (1941) sought for the best way of preparing alum precipitated diphtheria toxoid and found that the greatest degree of purity did not concide with best yield but choosing an appropriate concentration (1 5 per cent) of alum and proper *p*H of precipitation (4 9-5 0) a high degree of purity and yield was obtained. According to the authors the stability of the precipitate was enhanced by using 0.5 per cent dibasic sodium phosphate as the suspending medium. Basu and Sen (1941) found that ferric ammonium sulphate precipitates diphtheria toxin from the filtrates more effectively than potassium aluminium sulphate, the suspensions were, however, less antigenic '*in vivo*" than that obtained with potash alum Yield of toxin could be favourably influenced by addition of D. lactic acid in the culture medium, as reported by Sen and Basu (1945). Mirdamedi and De (1946-47) investigated the possibility of quickening the flocculation of toxins and antitoxins by addition of adjuvants to the mixtures Emulsion of tolu balsam when introduced was found to hasten the reaction in diphtheria toxin and antitoxin systems. Stability of antitoxin was studied by Goyal (1948) and, when tested against one particular toxin, deterioration to the extent of 15-37 per cent per annum at room temperature (45° — 86°) was noted. Basu and Roy (1946) tested the incidence of natural diphtheria toxin in horses and showed that 65 per cent of the animals had less than 0 02 unit per cc, 10 per cent had between 0 02 and 0.1 unit per cc and 25 per cent had 0.1 unit or more Among such horses which showed appreciable response to primary diphtheria immunising stimulus, however, there was, irrespective of the titre of the naturally occurring antitoxin, no significant difference in the final titres obtained. De and Basu (1938) in their pioneer work pointed out the

beneficial effects of combined (Sulphanilamide and antitoxin) therapy in bacterial infections in laboratory animals. Almost simultaneously British workers published papers which supported the claims of the authors.

Most works on clostridia group centered round the study on the preparation of potent toxoid particularly of tetanus and also on the reaction of toxins and antitoxins. Basu, Roy and Ghosh (1938) reported on Danysz phenomenon with *Cl. septique* toxin and antitoxin. Lahiri (1940) demonstrated that Ramon's flocculation test could not replace entirely the procedure of the estimation of antigenicity by direct inoculation into experimental animals. Earlier he (1938) reported that flocculation test for titration of tetanus antitoxin was not as effective as with diphtheria antitoxin He (1939) also extended a similar study to toxin and antitoxin of *Cl septique* In 1939, Basu (1939) described a flocculation test with *Cl septique* toxin and antitoxin The results were compared against those obtained by the guinea-pig internormal method. The same author (1943) further worked on flocculation test with *Cl oedomaticus* toxin and antitoxin and found results which were fairly reproducible when tested on animals.

With regard to immunity in tetanus, Lahiri (1942) immunised 23 volunteers with tetanus-formol-toxoid following British and U S.A. army schedule and found the U S A. method better He (1942) also claimed that mice might be used for titration of prophylactic tetanus toxoid by protection test. Pashricha and Panja (1940) recovered *Cl. botulinum* from four out of eight stray samples of garden soil at Calcutta Toxin elaborated by all four behaved like botulinum toxin Type A The implication of the finding with regard to botulinum in India has been discussed. Basu and Sen (1941) have described a peptic digest broth for the production of tetanus toxin Toxin just produced was used by them on 200 horses for immunization without allergic symptom in any one of them. In the same year they also described a media for the preparation of *Cl. welchii* toxin, in which peptic digest veal was added to veal infusion in suitable proportion. The media yielded a toxin of good antigenic value Seal (1942) described a modified liver and veal digest broth for the production potent toxin (Type A) of *Cl. perfringens*. He further described that perfringens toxin is essentially composed of two components characterised by two distinct types of hemotoxin.

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Pasricha and Panja (1940) reported results of examination of 300 throat-swabs from apparently healthy individuals The result of these swab cultures showed that in 54 or 18 per cent of the cases cultured haemolytic streptococci were present. Thirty-three of these 54 strains, i e., about 10 per cent of total 300 cases examined, belonged to Lancefield's Group A. Alpha variety of streptococcus was present in 57 per cent of cases, *Haemophylus influenzae* in 52 per cent and *Streptococcus viridans* in 47 per cent of the total cases Chatterjee and Mitter (1941) isolated 92 strains of haemolytic streptococci from throats of apparently healthy persons From culture of cervical swabs from 30 consecutive patients on the third or fourth day after delivery, they obtained haemolytic streptococci in 61 cases and concluded from this study that Group A strains of haemolytic streptococci were responsible for the majority of severe puerperal infections A febrile patient showing haemolytic streptococci other than Group A has been observed by these authors (1941).

Study on the classification of Staphylococcus was made by Goyle and Minchin (1940) From biochemical and serological data on 101 strains of staphylococci, they found that 76 per cent of pathological strains were both pigmented and coagulase positive, 76 per cent of the saprophytic type were non-pigmental and coagulase negative. 90 5 per cent of the staphylococci from pathological sources were either pigmented or coagulase positive.

Taylor and Chitkara (1940) investigated bacteriologically 284 cases of lobar pneumonia and 16 cases of broncho-pneumonia. Thirty-seven per cent reported to be Type I pneumococcus. A comparative study on the capsular reaction and agglutination test in the typing of pneumonia made by Pandalai (1942) demonstrated only Type I and Type II.

Dharmendra (1940) made a study on the viability of meningo-cocci at incubator temperature and in cool room (3½°C to 8½°C) Two media were used, the pigeon blood agar slants and semisolid serum agar media. All six of 35 strains, which belonged to Group II (Griffith), survived in cold room for 31 weeks on the former and 27 weeks in the latter. None of the other strains survived beyond 8 weeks under any condition

Study on the transmission of anthrax through flies was made by Sen and Minett (1944). *Stomoxys calcitrans* failed to transmit anthrax either by its bites or through infected excreta But *Musca domestica* and *Calciphora erythrocophala* could do when brought in contact with cauterised skin of normal goats. Bacilli could be found in the bodies of such infected flies up to 72 hours and in the faeces between 21 and 32 hours. Possibility of dissemination of anthrax infection through dirty, stagnant pools have been stressed by Naik (1938). These findings, if

scientifically established, would be of great importance, since it will probably be the first instance in India that stagnant and contaminated pools should be incriminated sources of natural infection by anthrax

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Much work on typhus group of infection was concentrated on the reports of clinical infection. During the later part of the decade under review attempts for the isolation of the organism *in vitro* were made. But the outstanding work was done by Smith and Krishnan, regarding the mechanism of transmission of the infection through the arthropod vector.

Reports on clinical infection:

Yacob (1937) reported an outbreak of typhus fever in a village of Muzaffargarh district, S.W Punjab with clinical reports of 11 cases which on investigation gave positive Weil-Felix reaction for Proteus OX 19 (in 6 out of 7 cases tested) but in low dilutions, Proteus OX K was not agglutinated Kapila and Moitra (1937) reported 1 case of fever lasting three weeks A positive Weil-Felix reaction with OX K strain was obtained on the 15th day of fever. Hassett (1937 reported a severe case of fever with delirium and unconsciousness giving positive Weil-Felix reaction in a dilution of 1/1000 for Proteus OX 19 and OX 2 Sarker (1939) published a case report with fever in a European in which Widal reaction was negative but Weil-Felix was positive for Proteus OX 2 with a rising titre and was negative for OX 19 and OX K

Sharma (1940) in Bangalore tested all cases of fever giving negative Widal reaction or negative blood culture for the Weil-Felix reactions. 56 cases gave positive reactions for typhus fever, 50 for Proteus OX 19, 5 for OX 2 and 1 for OX K In the majority of the cases the fever lasted for 10 or 18 days and typical rashes were seen in 12 cases The vector of endemic typhus in Bangalore has not been determined, but the majority of the cases occurred among poor people in the city.

Heilig and Naidu (1942) reported 14 sporadic cases in Mysore City, four cases being from villages in the States 4 cases occurred in August, 2 in September and 1 in each of the months of October to February. There were no cases in hot months, March to July. Weil-Felix reaction was negative in six and positive in remaining 8 cases. There was no louse infestation in any case and no history of bites by ticks or mites could be obtained. All the patients, however, lived in rat-infested areas and in close proximity to tick-infested cattle In a subsequent report these authors (1914) extended their report on typhus infection in Mysore State and gave an analysis of Weil-Felix reaction in 18 patients, OX 2 titre was 1/80 or over in 16 patients and OX K titre was 1/80 or over in 6 patients. The disease was regarded by the authors as being probably tick-borne and as being serologically and clinically identical to Boyd's X 2 typhus.

Singh (1943) reported one case having typical clinical feature of Indian tick

typhus The Widal and Weil-Felix reactions were negative The place of occurence was Meerut and it was the third case reported by the author during 30 years in Meerut The case was reported to be a clinically typical example of the tick typhus in India and the epidemiological evidence strongly supported this diagnosis

Twenty-six sporadic cases of endemic typhus occurring in Bombay City has been reported by Patel (1943) that Rickettsia have been obtained from one of such patients by inoculation of blood into the guinea-pig The proteus OX 19 titres ranged from 1-50 in one fatal case, it was 1-250 or 1-500 in the other cases The authors believed that all the conditions for a serious epidemic of louse-borne typhus existed in Bombay

Sen Gupta (1944) reported a case of Kala-azar who after completion of a course of specific treatment developed dusky erythematous blotchy rash and fever Proteus OX K was agglutinated by his serum at a titre of 1-400 on the 7th day of the illness, the titre fell to 1-25 on the 10th day and reaction was negative on the 14th day Neither Proteus OX 19 nor Proteus OX 2 was agglutinated The type of the agglutination response was regarded as confirming the diagnosis of typhus caused by a strain of rickettsia similar to that of the *tsu-tsugamushi* fever.

Lowe (1946) reported 10 cases of typhus infection seen in 1945 at the School of Tropical Medicine, Calcutta Only one case was previously reported in 1944 and one scrub typhus in May, 1946 The diagnoses were based chiefly on the serum agglutination reactions, four were diagnosed as scrub typhus, four as murine typhus, one as tick typhus and the remaining cases were of doubtful etiology

One hundred twenty-one cases of "typhus fever *(OX K)" occurring in a British* regiment during October and November, 1945 in an area adjacent to Burma were reported by Tattersall and Parry (1945) The Weil-Felix titre for OX K usually *reached 1-200 by the 13th day.*

Lusk (1945) reported 140 cases in an Indian Military Hospital in Calcutta. The author saw the cases during the months of June to December, 1943, 60 from a village and 54 from Calcutta *The great majority of these cases could be* assumed to have been mite-borne, there might have been 12 flea-borne and possibly one or more tick-borne cases Louse transmission seemed to have been excluded. He broke up *his data in the following way* ·

Serological type	Village cases	Calcutta cases	Total fatal
OX K	55 (4 fatal)	41 (7 fatal)	11
OX 19	3 —	9 —	0
OX 2	0	1 (fatal)	1
Clinical and post-mortem evidence	2 (2 fatal)	3 (3 fatal)	5

On Isolation, diagnosis and epidemiology:

Studies on diagnosis and epidemiology of the Rickettsial infection had earlier met with *frustrations and failures because of the wrong assumption* (on the basis of Weil-Felix reaction) that Proteus group of bacilli had some etiologic relation to the typhus group of infections. Inspite of negative results, works pursued on this line will nevertheless be reviewed not only for their intensive scientific value *but as an example of zeal* exhibited by so *many* workers in India to solve one of the besetting problems of life in India

Fevers of the typhus group attracted the notice of some workers from 1937-43. Thus Smith and Mehta (1937) worked to isolate OX 19 and OX K from rats and failed to effect experimental transfer through fleas from rats to guinea-pigs, though sera of the rats showed high titre and clinical cases of OX K were occurring Webster (1940), Shortt *et al* (1940) and Goyal (1941) attempted to isolate OX K strain Webster failed but Shortt, Pandit, Anderson and Sanjiva Rao *demonstrated that infection agent could be successfully cultured from blood of* experimental and clinical infections on chorio-allantoic membrane of eggs. They could also transfer infection to normal experimental animals Their attempt to immunize normal volunteers with vaccine prepared from the culture developed certain degree of immunity in the recipients Goyal was also successful in isolating rickettsial bodies from rats in Calcutta and induced mild experimental infection in guinea-pigs Rat strains seemed to be non-pathogenic to man Later mostly through the work of Krishnan and his group, culture of the rickettsia in fertile egg and repeated passage through generations in chick-embryos were successful. Passage through chick-embryo enhanced virulence for mice, while passage through mice enhanced virulence for guinea-pigs (1948).

Works on isolation and culture of Rickettsial bodies in laboratory was mainly carried out at two centres, — Bombay and Calcutta — under the directions of Drs. D. W. Soman and K V Krishnan respectively They have reported isolation of rickettsial strains (*R orientalis*) from clinical cases of human infections (1947) On the basis of their work, diagnosis of Rickettsial infections in India has become less problematical. Animal-inoculation of infected blood in white mice has become a very helpful procedure and is at present practised as a routine method in suspected cases, according to the opinion of Krishnan (1948) none of sero-positive cases (in his study) gave positive results by this method. *Furthermore, using the Rickettsial strain* cultured in the laboratory, serologic diagnosis of the infection has been put on a specific level It has, however, been expressed that local strains would be more reliable antigen for complement-fixation and agglutination reaction than the stock-strain.

With a view to discovering the vertebrate reservoir and arthropod vectors, responsible for the transmission and maintenance of the endemicity of the human

infection, various spices of rodents (in areas where clinical infections are endemic) and their ectoparasites were studied for the presence, in them, of the infectious agents and for effecting successful transfer of the infection, when available evidences suggested its presence, from such infected reservoir to normal healthy recipients in the laboratory.

Report by Mehta (1937) on an investigation of 2,451 rodents, rats, mice and shrews (1935-36) with reference to the ectoparasites in relation to the seasonal variations, brought out an interesting point. At the time (cold weather) when the maximum of the murine typhus cases (OX 19) occurred, the flea population was at its minimum on the rats, and when the (OX K) cases occurred in August, September and October just at the end and after monsoon, fleas, lice and ticks on rats and other rodents were at their maxima The possible role of fleas, mites, ticks and lice in the transmission of typhus in the Simla Hills was discussed and it is emphasized that the larval mites and the *Hyalema tick* might possibly be concerned in the transmission of the rural type (OX K) of typhus.

Investigation by Smith and Mehta (1937) has revealed that of the two waxes of typhus met with in the Simla Hills — one in the winter and the other occurring just after monsoon in August, September and October, the former, i.e., sera of typhus occurring in winter agglutinate Proteus OX 19 sera of the latter agglutinate Proteus OX K. They carried out researches with a view to the isolation of the virus of the OX K variety and the carrying out of transmission experiments with ticks and mites Sera of 1,212 wild rats captured from May, 1935 to February, 1936, were tested for agglutinins It was observed that there was a marked increase during the months of October, November and December of sera which agglutinated OX K in significant dilutions. Two of the rats showing agglutinin in high dilution for OX K were selected and mites *L. bacoti* were collected from them and emulsified and injected into guinea-pigs but without results

Fleas of the genus *Nosopsyllus* (*Ceratophyllus*) were fed on these rats and later emulsified and injected into a normal guinea-pig but without result. A nymph of the tick *Rhipicephalus sanguineus* was found on one of the rats. It was allowed to grow and later removed. The adult tick was emulsified and injected but without result The rats were then killed and the brains and spleens removed and injected into guinea-pigs but without result

With a view to seeing if any change resulted in the nature of the virus (OX 19) when transmitted by ticks or mites numerous ticks and larval mites were fed on guinea-pigs and rats infected with the local X 19 virus; they were then emulsified and injected into other animals but without producing any infection. It is interesting to note that when fleas *N. simla* were fed on the same infected animals

later injected into guinea-pigs they produced the typical infection of a murine virus fever and orchitis without production of agglutinins in a rabbit for OX 19.

In August and September when cases of the OX K type of fever were occurring and fleas were very numerous on the rats collected, fleas were taken from these rats, emulsified in batches and injected into guinea-pigs. A murine virus X 19 was isolated on three occasions but none of virus of the OX K types.

Investigation on the problem of typhus in the Simla Hills was continued by Webster (1940), and it was possible to isolate the Proteus X 19 typhus virus from rats caught locally. It was also found that the smaller types of local monkeys harbour large numbers of *Trombicula deliensis* larval mites, but so far it had not been possible to isolate any virus by inoculation of emulsions of these larval mites in guinea-pigs It was not possible to isolate a virus from the blood of the monkeys either and the Weil-Felix reaction on their sera was also negative; nor could they be infected by injections of the human of X K virus.

Goyal (1941) made a study of the 100 rats caught in Calcutta from January 1937 to December, 1938 and concluded that rat strain of virus was non-pathogenic for man 300 other rats were killed and examined during the period September, 1939 to July, 1940 but none was found to be infected. Heilig and Naidu (1942) from another study on 14 cases of the fevers of typhus group in Mysore observed that there was no louse infestation in any case and that they differed essentially from the "Bangalore type" which is supposed to be rat-flea typhus; clinically and serologically their cases closely resembled Boyd's "X 2" group, which is related to Indian tick typhus

Stoker (1948) studied incidence of murine typhus amongst wild rodents in Poona and Bombay by complement-fixation tests on their sera with specific antigen and found about 9 per cent positive results. In a similar study on rat population around Bombay, Soman and his associates (1948) gave a figure of 45 per cent positive results for *Rattus nervegicus*, about 19 2 per cent for *R. rattus* and none for *Suncus ceruleus*

Situation, as evident from above paragraphs, was very confusing until Smith, Krishnan and their associates at the All-India Institute of Hygiene and Public Health, Calcutta, solved, through their ingenuity and painfully patient research the mystery of transmission of this infection from vertebrate reservoir to human hosts through the ectoparasites of rodent population prevalent in the endemic areas. Crystallizations of their success was brought about by the successful method, worked out by these works, of breeding under laboratory conditions larvae of *Trombiculid* mites (predominantly *Trombicula deliensis*). Krishnan and his group (1949) later reported a successful transfer of Scrub typhus to non-infected laboratory mice through the agency of such laboratory-bred infected larval mites.

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VIRUS INFECTION

Very little has been done on virus group of infection during the decade under review. The work has mostly been on the immuno-protection particularly of rabies Attempts on the study of other group of viruses have been undertaken by very few investigators One of such was the report of Maplestone and Panja who showed in 1939 that filtrate obtained from the suspension of molluscum nodules could transmit infection to rabbits They could produce (1939) successful passage in series and suggested that the antigen prepared from such experimentally infected brain could be effectively used for diagnostic complement-fixation reaction with patients' sera A report on the presence of Psittacosis virus in parrot in Calcutta was contributed by Goyal (1940), Chatterji, Gupta and De (1945) published their observations on 89 cases of virus encephalitis with a high mortality, about 72 per cent mortality in older age group and about 87 per cent under 5 years of age.

Rabies

Rabies being a much dreaded disease, the problem of its immuno-prophylaxis gained a priority in the mind of workers in India during the last decade Several of these workers have put in sustained effort to work out a better and more potent vaccine for prophylaxis than what was in vogue before the period under review. Workers who will stand out on this point are Veeraraghavan and his associates working at the Central Research Institute, Kasauli All such investigators worked but with one motive i.e how to improve the potency and reliability of the standard vaccine used in anti-rabic prophylaxis and consequently were bent upon preparing a concentrated preparation of infective virus particles, since the prevailing idea was that the degree of immuno-prophylaxis would probably be a function of the viral content of the vaccine Earlier attempts at the culture of rabies virus on the chorio-allantoic membrane of growing chicken by Veeraraghavan and Philipsz (1938) were unsuccessful, though these authors (1940) reported a successful experimental infection of rabies (isolated from a rabid jackel) in domestic fowls. Subsequently, however, Veeraraghavan (1946 & 1947) claimed to have succeeded in culturing rabies virus in a cell-free enriched medium containing sheep brain extract Concentration of virus particles in such "cultures", according to the author, was about 3,300 times that ordinarily obtained in infected brain then used in the preparation of anti-rabic vaccine. He (1945) claimed that his technique of "culture" would provide not only a rapid and delicate method for diagnosis of rabies in animals, but could also afford, when used as vaccine, considerable degree of protection, which was in animal experiments as good, if not better, as that with phenolised vaccine (1946). It has also been claimed that 1 per cent vaccine from such "culture" was poorer in its protective potency than that of 5 per cent strength

(1947). Fixed virus in such "cultures" was not filtrable and when stored with 50 per cent glycerol at 0°C, it retained its infectivity even for 5 months (1948). Dogra (1948) made a comparative study on the protective efficacy of 5 per cent Semples' sheep brain vaccine and 25 per cent phenolised Savoors' sheep-brain vaccine. He claimed that Semples' vaccine was superior to Savoors'. Dogra in collaboration with Sanjiva Rao (1948) further showed that 5 per cent Semples' vaccine was uniformly effective in mouse protection tests and 2 per cent vaccine (as used routinely in Bengal) was of poorer potency than the 5 per cent variety. Goyal (1948) reported wide variations in the protective potency of different antirabic vaccines prepared at the Central Research Institute, Kasauli and suggested a pooling of several preparations to ensure a standard and reliable immunoprophylactic quality of the vaccine For demonstration of Negri bodies in infected tissue, McDonald (1944) described a simple method.

Virus causing animal and human pox

Sanjiva Rao cultured sheep pox virus in chick embryo and had shown it to be definitely modified by such passage On inoculation the cultured virus, while inducing only a local reaction in sheep, appreciably protected against subsequent infections with natural virus (1938) Pandit and Sanjiva Rao (1940) made a comparative study on two strains of vaccine viruses, one from small-pox-material and other the strain used for the production of vaccine lymph. Protection tests on actively and passively immunised rabbits suggested a close similarity between these strains, which was further confirmed by agglutination, complement-fixation and virus-neutralisation tests

Reports on the clinical aspects of small-pox have been communicated by several investigators Panja and Das reported successful treatment of confluent variola with antigen-antibody mixture on a small number of cases (1942) Vengasarker, Poonen and Walavalkar (1942) employed prontosil in the treatment of small-pox and concluded that its administration reduced mortality in confluent and semi-confluent cases with lesser complications during the course of the disease and convalescence. Number of cases treated was 639 (1942) Sen (1945) investigated 1,929 patients for the incidence of ocular complications in small-pox. Cases showing such complications in his series were 236. About 19.7 per cent were due to small-pox while the rest was non-specific infection Commonest non-specific organisms cultured were staphylococcus, Koch-Week's bacillus and Diphtheroid

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IMMUNOLOGY AND SEROLOGY

The new science of immunology and its twin serology had budded out of bacteriology only a few decades ago Yet, through the care and unsparing efforts of their nurses, the immunologists and the serologists, they have grown to full-fledged sciences. Indeed, they have grown so profusely as to cover the domains of parasitology, toxicology, epidemiology and clinical science. The Indian scientists have contributed their mite towards the development of the twin, not inconsiderably None can, indeed deny the effect of these contributions on the advancement of the science of immunology

Cholera : It is one of the infectious diseases which has caused havoc in India in the past, and still causes considerable mortality especially in the Ganges delta. Naturally, considerable efforts have been made for identification of the true causative organism and for preparation of a potent prophylactic for immunization against cholera

Careful studies of Gardner and Venkatraman (1935) revealed the antigenic structure of *V cholerae* They clarified the confusion prevailing previously, and concluded that the true cholera vibrio was a non-haemolytic organism containing the specific O antigen of sub-group I The paracholera or cholera-like vibrios, isolated from cases of choleraic diarrhoea or from water, belonged, unlike the cholera vibrio, to sub-groups II to VI This study, thus, made it possible for identification of true cholera vibrio on the basis of serological tests They were agglutinated by Inaba or Ogawa type of sub-group I serum. So also were the EL Tor vibrios, but they were haemolytic to sheep or goat blood cells

Similarly, the immuno-chemical studies on vibrios by Linton and his colleagues (1928, 1936, 1938, 1938-1939) have classified them into six groups, not quite related to the serological groups of Gardner and Venkatraman Basing the identification on the relative content of two different proteins and three different polysaccharides of these vibrios they were classified into six groups. True cholera vibrios were scattered among chemical groups I, II, IV and VI.

Efforts of preparation of a potent cholera vaccine seems also to have borne fruit. The new casein hydrolysate cholera vaccine is claimed by Sokhey (1950) to be much more potent than the agar grown vaccines. In laboratory animals, of course, the vaccine has proved to be superior to all other. If these results were repeated in the field, a definite advance would have been made in immunization against cholera.

However, even the less potent agar-grown cholera vaccine has shown its worth in the prevention of cholera Field enquiries by Adiseshan *et al.* (1947), and Chandra Sekar (1947), have shown that inoculation with this type of vaccine lowered the attack rate 50 to 80 per cent, though the case fatality rate showed no

significant difference between the inoculated and uninoculated population. They also found in these field trials that the immunity lasted for at least 6 months, and perhaps might last for 12 months or more

Plague · Though the frequency of epidemics of plague seems to be waning now, the disease is still endemic in many parts of India. Any apprehension about its outbreak still produces terrible scare It is, therefore, natural that most of the outstanding contributions on plague have been made by Indian workers, especially of the Haffkine Institute, Bombay

Sokhey (1939) established that the strain of white mice inbred in the Haffkine Institute was highly susceptible to plague and evolved an exact method of determining the virulence of plague strains This virulence could be maintained indefinitely by drying the strains from the frozen state. With the virulent strains, Sokhey evolved methods of assaying the immunizing potency of plague vaccine and antiplague serum in white mice (Sokhey *et al* 1950).

These animals were as a rule fully protected when immunized with 0 03 ml of Haffkine vaccine, while 10 times this amount was required to produce toxic death in these animals (Sokhey and Maurice 1935)

Recently, Sokhey has evolved a new casein hydrolysate vaccine (1950). It proved to be about 10 times more potent than the Haffkine vaccine, when both were compared in white mice For, the mouse-protective dose of the new casein hydrolysate vaccine was 0.004 ml (that of the Haffkine vaccine was 0 03 ml). The toxicity of the new vaccine was also much lower, about one-third of the Haffkine's (Sokhey and Habbu 1946)

Haffkine Institute has also evolved a new anti-plague serum derived from hyperimmunized horses The mouse-protective dose of this new serum was on the average 0 05 ml (Sokhey 1936).

Development of natural immunity against plague was also an interesting observation Rats caught from a plague infected area showed themselves to be highly immune to the challenge of virulent strains. Sokhey and Chitre (1937) found that the proportion of resistant rats corresponded closely with the total human plague incidence in the town in which both the rats and the men lived Thus, in Bombay city, where plague had been prevalent, only 7 9 per cent of the rats died after inoculation of a standard dose of virulent plague bacilli; in Madras city, as a contrast, where there had been practically no plague, 91.1 per cent rats died after similar challenge

Plague vaccine has been used extensively in India for immunization against plague. Sceptics had, however, several occasions to raise scare against its use Soon after Haffkine introduced his vaccine, some critics raised the bogey of negative phase following vaccination. If negative phase did really develop, the inoculated

would have been more vulnerable to plague till a few days after plague vaccination. (Patel and Rebello 1948). The results of mass inoculations with Haffkine's vaccine, however, proved the contrary, "there was no adverse effect observed among those inoculated after exposure to infection".

Just as plague vaccine seemed to control incidence of plague, anti-plague serum showed itself to be able to reduce the mortality in the infected. The effect of serotherapy seemed, however, to depend largely upon the absence or presence of bacteraemia.

Serological tests, however, could hardly distinguish between virulent and avirulent, smooth and rough strains (Wats *et al* 1939). Nevertheless, a slide agglutination test has been found useful by some workers for diagnosis of human plague (Panja and Gupta 1949).

Enteric infections : Immunology and serology of enteric infections do not seem to have received much attention of Indian workers though heavy rates of morbidity and mortality from these diseases are returned by health authorities A significant contribution was, however, made by Bhatnagar (1935), who in association with Felix, found out the Vi antigen of virulent *S typhi* This antigen stimulated the development of Vi antibody in men and animals Presence of Vi agglutinin, however, is seldom of any diagnostic use, but the titre of Vi agglutinin is claimed to have prognostic significance. Bhatnagar (1944) reported that in severe cases of the disease Vi agglutinin was absent, or present only in low titre, while on the other hand, the cases, in which the Vi titre was high — 1/50 to 1/250 — and is maintained high for a number of days, were likely to recover rapidly.

Typhus Fever : Diagnosis of rickettsial infections through serological methods [illegible] antibody titres significant for diagnosis of the two common rickettsial [illegible] — scrub and murine typhus — in India, deserves mention They find that OX 19 agglutinin titre should be very high to signify diagnosis of murine typhus The OX K agglutinin titre, however, is considerably lower, and, hence, would signify diagnosis even if the titre were fairly low Workers in India have confirmed the opinion abroad that more reliable method in diagnosis of infection would be the complement fixation or the agglutination test dose with local strains (Report of ICMR 1947).

Diphtheria . Prevention of diphtheria has not received much attention in India, though the incidence seems to be rising gradually. It contains a natural adjuvant derived from the impurities of the crude toxoid, produced in veal-infusion-proteose-peptone broth. Both the toxoid and the adjuvant are purified from the same crude toxoid, and are then mixed up together This preparation, called N.A.F.T., has shown to produce in Indian children as good a response as is produced by A.P.T.

The chief advantage, however, seemed to be the absence of any untoward reaction following infection of N.A F.T.

Natural immunity of a high order seemed to exist in Indians against diphtheria. Studies in Bombay by Lahiri (1941, 1943) and in Calcutta by Ghosal (1941) have revealed presence of significant antidiphtheric immunity, especially in adults, grown-up children, and children living in crowded unhygienic habitations.

Tetanus Unlike diphtheria, and other diseases caused by toxins, immunity to tetanus did not seem to be acquired *in nature*. No tetanus antitoxin could be detected in a number of persons highly exposed to the risk of tetanus infection Nevertheless, very effective immunity could be induced in man with tetanus toxoid, and Lahiri (1942) found that three doses of 1 ml each of the toxoid, given at intervals of 4 weeks, produced better antitoxic response than two doses of 1 ml each given at an interval of 6 weeks.

Standardisation of immunological reagents The new lyophilised polyvalent anti-snake-venom serum of the Haffkine Institute, which is effective against all the four common poisonous snakes of India, has been standardised The serum is tested in white mice against standard venoms of these four different kinds of snakes.

During the earlier days of production of tetanus toxoid its assay was a problem. To meet this problem, a mouse protection test was evolved by Lahiri (1942). Similar tests were later recommended by American and Canadian workers

For biological assay of cholera vaccine, a method has been evolved by Sokhey (1944) This method consists of matching the unknown against a standard vaccine in mice, which are challenged, after immunization, with virulent organisms with mucin

Leprosy A refined and standardised protein antigen has been prepared by Dharmendra (1943) from dried and partly defatted leprosy bacilli. This preparation showed itself to be superior to the crude preparations in many respects. This refined product has, therefore, been preferred to crude preparation for "Lepromin" tests of leprosy.

Rabies · For the prevention of rabies, Semple's carbolised 5 per cent. suspension of infected sheep's brain still holds the field Nevertheless, alternatives have been suggested from time to time Veeraraghavan (1941), for instance, claimed preparation of antirabic vaccine in cell-free culture medium and reported it to be more suitable than Semple's vaccine Dogra (1948) lyophilised Semple's vaccine, and claimed that it possesses several advantages over liquid Semple's vaccine

Kala-azar: In addition to Chopra's test and aldehyde test for kala-azar, a complement fixation test for kala-azar was evolved with W.K.K. antigen. The diagnostic value of this test is said to be very high.

Blood groups : Surveys on the distribution of A and B antigens of red blood cells of different communities and castes in Calcutta have revealed prevalence of one group or the other amongst them. Similar surveys on distribution of M and N antigens have also been made on a smaller number of Calcutta population

Antigen-antibody reaction Theoretical considerations of the mechanism and the effect of environmental factors on the velocity of reaction have also received attention.

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IV. SURGERY

General Surgery

Andreasen (1941) described a new technique for operation of 'elephantiasis scroti' The scrotum was split into two longitudinal halves by an incision along the median raphe, the testicles with the cords are isolated, important vessels ligated at the anterior and posterior corners of the two scrotal halves and thereafter the affected portions of scrotum were removed with ease. There was need for minimum ligations of vessels and the operation could be completed almost unaided in about 45 min. Ramchandra Rao (1946) in a valuable paper discussed the anatomy of the various plantar spaces, the tendon sheaths of the foot and the leg by injection experiments and compared the fascial spaces in hand and foot This exhaustive paper contains many practical applications of anatomical findings.

Sen (1947) in a valuable paper 'on the problem of infection in burns' brought out many important factors responsible for infection in burn cases and their practical application for prevention and control of sepsis. These factors in order of importance are

(i) Depth of the burn, (ii) area, (iii) contamination, (iv) duration before treatment, (v) site of the burn and (vi) the agent

He also compared burns treated with or without local sulphonamide Sulpha-treated cases had a mortality of 2 86 per cent and those without 5.5 per cent. Relation of blood protein to infection was estimated and it was found that low protein levels increased infection to a marked extent

Nat and Sachdeva (1947) described a standardised technique for rhinoplasty.

Mehta (1950) described the method of controlling filarial oedema by intra-arterial glycerine injection, 25 to 50 per cent sterile glycerine solution in normal saline are injected intra-arterially once or twice a week followed by 20 to 25 intravenous injections Of 70 cases treated, in 22 the calf circumferance was reduced by more than 2", in 27 by 1 to 2", in 10 by less than 1" and in eleven there was no change He also described a new operative method for elephantoid legs by dissecting out large flaps of skin over the elephantoid tissue and replacing them after excision of the filaria-laden tissue.

Kini (1944) reported on a case of carcinoma penis in a child of 2 years This was a papillary growth infiltrating the wall of the corpus spongiosum.

Bhaskara Menon and Sheppard (1944) described 4 cases of lymphadenoid goitre showing an increasing extent of involvement of the glands From a histological study it was suggested that there was a sequence of changes from lymphoid hyperplasia, germinal hyperplasia, diffuse lymphocytic and plasmacytic infiltration with destruction of vesicular structures to increased reticular formation and fibrosis.

Frimodt Moller (1945) in investigating colonic movements in Indians and Europeans during barium-meal examination found that there is not much difference in the rate of progress of barium-shadow through the colon except after 24 hrs, when Indians showed a definitely faster movement than the Europeans The average time for barium-meal to reach the hepatic flexure in Indians was about 3 hrs, splenic flexure 4 hrs, descending colon 6 hrs, and the iliac colon 8¼ hrs, the relevant figures for Europeans being 4¼, 5½, 8½ and 10¾ hrs

Khanolkar (1945) in a study of the clinical material from medical institutions in India as well as a guarded use of official statistics suggests that Indians are as liable to suffer from cancer as the inhabitants of western countries

Bose and Mukherjee (1944) made a comparative study of the anaesthetic action of trilene, trichlorethylene, chloroform and ether on mice, guinea-pigs and rabbits In anaesthetic properties trilene and trichlorethylene showed certain characteristics common to both chloroform and ether, but muscular relaxation was almost as imperfect as with nitrous oxide Both trilene and trichlorethylene are less toxic than chloroform but ether proved to be safer than either of them The anaesthetic dose of trilene and trichlorethylene is slightly greater than that of chloroform but is much less than that of ether. Favourable features of trichlorethylene anaesthesia are its rapidity of action, its analgesic effect and relative absence of early fatal accidents. Its disadvantage is its failure to produce muscular relaxation. In view of observed toxic effects on the kidney and lung after heavy doses of trichlorethylene it should not be used for prolonged anaesthesia.

Goyle (1950) reports a case of combined carcinomatous and giant-celled sarcomatous growths in the same breast in a woman aged 52 This was the 7th of such cases recorded in the literature.

Abdominal Surgery

Somervell (1940) from his experience of over 3,000 cases of peptic ulcers in south India came to the conclusion 'that deficiencies in vitamins specially vitamin A is the chief dietetic cause of duodenal ulcers' His colleague Janorr from his researches came to a similar conclusion and added that deficiency in proteins and vitamins A and B brought about changes in the myenteric plexus of stomach and duodenum resulting in spastic, secretery and congestive changes in the organs At the same time, the lack of protective vitamin A lowers the resistance of the mucose to infection and leads to lymphocytic invasion of the mucose and hyperplasia of lymphoid follicles which eventually rupture into the surface forming microscopical ulcers. The effect of hypersecretion, hyperperistalsis and spasm is to delay or prevent healing of these ulcers which eventually coalesce to form one large macroscopic ulcer.

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Somervell also elaborated the operation of vascular ligation previously described by Wilson Hay and called it 'physiological gastrectomy' The operation in his hands has been followed by prolonged reduction of acidity with benefit to the patients.

Dogra (1941) investigated the incidence of peptic ulcers in different parts of India and reported the following figures for the incidence per 1,00,000 per cent of the population ·

Madras	—	143
Bihar	—	37
Bengal	—	33
Orissa	—	29
C P. and U.P	—	10.12
Assam, Punjab and Bombay	—	1.9

Chitre (1944) studied 24 cases of proved cancer of the stomach for the gastric secretion after an alcoholic test-meal. Out of these 12 cases presented complete anacidity and in 9 cases acid secretion was greatly reduced In 3 cases acid value was quite high.

Nigam and Sircar (1945) described a red — 'Cholesterol tolerance test' for helping in the diagnosis of chronic cholecystitis. They found that by giving a comparable quantity of cholesterol by mouth to patients with chronic cholecystitis the rise in the serum cholesterol was more than 100 mg on the average while in normal people such rise was no more than 52 mg

Joshi (1945) in a valuable paper worked on the aetiological problems of urinary calculi and concluded that the uriniferous tubules probably set on the principle of a flush, that all deposits in the kidney which are insoluble (pigments and casts) could be seeds of urinary stones, that sodium chloride and sulphates have the property of preventing the formation of urinary stones They also found that tertiary rock map of the world in association with the big rivers resembles the map of distribution of urinary stones; that scarcity or abundance has no effect on the incidence of stones, that temporary hardness of water is one of the most important causes, and that town people get more urinary stones than the rural population if the water is not chlorinated or filtered.

Mahadevan (1947) described the treatment of pancreatic pseudocysts by primary anastomosis to the stomach or jejunum and considered this to be a better method than the time-honoured method of 'marsupialisation'. Of 4 cases treated by this new method 3 were doing well for periods up to 3 years.

Banerjee (1950) in discussing causes of acute small bowel obstruction came to the conclusion that volvulus of small intestines or more correctly twist of the mesentery was the commonest cause in his series. This produces obstruction in

continuity by the doubled-up mesenteric border pressing on the terminal ileum, or the caecum or the ascending colon. This is not a loop obstruction and consequently strangulation or gangrene of a loop never occurs in this condition. The pressure on the bowel at the site when it is crossed by the mesenteric border leads to ischaemic condition varying from a pressure mark to a necrotic band.

Orthopaedic Surgery

Narashiman (1940) described the affections of bones and joints in small-pox and described 16 cases He noted epiphyseal, periosteal, medullary and arthritic lesions. Circumferential and longitudinal growths of bones were arrested in some cases There was predilection for the bones and joints of the upper extremity especially the elbow joint.

Ananthanarayana Ayer and Sitarama Rao (1941) determined the 'carrying angle' of the elbow in south Indians in a series of 20 cases and concluded that the mean value was 166° The range in adults varied between 155° to 174.25°, the majority lying between 160° to 170°. The value of the angle on the right side was larger than that on the left side by about 4°

Basu (1948) worked on the aetiological factors for the maintenance of the longitudinal arch of the foot and concluded from, experimental, clinical and embryological investigations that the longitudinal arch of the human foot is a permanent and rigid structure brought into being according to the tenets of 'Walff's-law' because of man's terrestrial habits and upright posture. For the appearance of the arch the increase of the size of the heel, its inclination, increase in the tarsal section of the foot, the diminution of its forepart, and increased strength of the medial side of the foot are responsible, for its maintenance the bony configuration, and the ligamentous supports are the important factors The contractural tone of the muscles may be of some help during locomotion but the postural tone is ineffective.

Surgical Anatomy

Narashiman and Bhaskaramurthi (1942) investigated the age of appearance of secondary centre in the lower end of humerus in Madras Presidency and their findings were as follows

Capitellum	—	1 to 3 yrs
Medial epicondyle	—	3 to 11 yrs
Trochlea	—	9 to 13 yrs
Lateral epicondyle	—	9 to 14 yrs

Ananthanarayana and Bobjee (1943) investigated the length and mobility of pelvic colon in south Indians and concluded that the pelvic colon in south Indians

are relatively short, the average length being 9" Making allowance for exceptions, as a rule, its range varies from 4" to 13". The depth of its mesocolon from its root to its colic attachment is on an average 2½" with an usual range of 0" to 4".

Appajee (1945) investigated the anatomy of the inguino-hypogastric and inguino-femoral regions and came to the following conclusion:

(a) The muscles-interval oblique and transversus have attachments along the whole length of the inguinal ligament, even when they are non-muscular, one can trace their aponeuroses to the ligament.

(b) The fascia transversalis is not the muscle fascia of transversus abdominis but it must be considered as the membranous layer of extra-peritoneal fatty tissue.

(c) There are 4 openings in the anterior abdominal wall for passage of testis and cord Two of them are named the internal and external rings. Intermediate ones in inner two muscles are not named

Experimental Surgery

Strain (1941) discussed the use of cotton thread as a suture material and concluded that cotton is an inexpensive, easily prepared suture with low incidence of infection and a predictable period of known strength in all patients In a series of 101 patients, cotton sutures only being used infection rate was only 3 per cent.

Andreasen (1941) conducted experiments to test the nature of the thoracic duct fluid after intestinal strangulation and attempted to use the drainage of the duct as a therapeutic measure He concluded that normal thoracic duct fluid does not affect the B P tracing, that the fluid in cases of intestinal strangulation depresses the B P tracing temporarily, that the depressor effect was not due to contained haemoglobin Fistulisation of the duct was followed immediately by opening up of left to right lymphatic anastomosis Cats with fistulae did not survive appreciably longer than those without fistulae; drainage of the duct therefore did not appear to be a rational therapeutic procedure

Wahid (1941) conducted a series of animal experiments to work out the aetiological factors of urinary calculi The effect of obstruction, infection and foreign body were noted both in upper and lower urinary tracts. In the upper urinary tract obstruction all the animals showed hydronephrotic atrophy and none showed primary atrophy Infection led to generalised pyelonephritis In the lower urinary tract obstruction showed fibrosis and trabeculation in the bladder wall. Foreign bodies in the bladder provided ready-made nucleus for the colloids to deposit.

Neurosurgery

Balkrishna Rao (1945) performed prefrontal leucotomy in 50 cases of psycho-

logical disorders — 11 cases recovered, 15 improved, 7 were unchanged, 5 cases were readmitted, 3 died and 1 was untraced. He designed an original and simplified leucotome.

Ginde and Cooper (1948) discussed the symptomatology, investigation and the treatment of 25 cases of spinal tumours. The horizontal interpedicular distances were measured and compared with a standard by the erosion and flattening of the pedicles. This distance differed in Indian patients from the American standard and during this work an Indian standard was laid down and was of some help in the investigation of the cases

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V. CLINICAL OBSTETRICS AND GYNAECOLOGY

During the period under review fundamental researches in obstetrics and gynaecology have been rather few. However, several important contributions in clinical obstetrics and gynaecology have been made Nevertheless, correlation of the findings of the different contributors is a difficult proposition, as in most of the publications selection of cases have been made on a somewhat arbitrary standard set up by each worker.

Diagnosis of pregnancy — The value of biological tests in the diagnosis of pregnancy has been established since 1929, but the difficulties of the general use of Aschheim Zondek Friedmann or rat test on a wide scale in India are obvious. Bhaduri (1950) employed the Galli-Mainini reaction on the Indian species of Rana and Bufo and observed that a correct diagnosis is possible in 94 per cent of cases. This work has been confirmed by Mohanti and Pabrai (1950) on *Bufo melanostictus.*

Erythroblastosis foetalis — Compared to western countries the work done in India for the period under review is meagre. Sporadic investigations have been done only in the large cities. Isolated case reports which provides no knowledge of the incidence of the condition have been published from other places. Sanghvi and Khanolkar (1947) reported 6 cases from Bombay and studied the incidence of Rh negative population as well as that of erythroblastosis The latter author also studied the incidence of rhesus negative persons among Parsees, Indian Christians and Marathas of Bombay and observed that in these communities 8 per cent, 6 per cent and 1 5 per cent repectively were rhesus negative (Khanolkar, 1948). On the basis of these figures Khanolkar (1948) states that the expected incidence of erythroblastosis foetalis in Bombay is 0 7 to 2.5 per 1,000 births. Ranganathan (1947) observed the incidence of rhesus negative population in Madras to be 4.1 to 7 per cent. Greval and Roy Choudhury (1946) found that 8 to 10 per cent of Bengalee population were rhesus negative. The importance of a study of the subject on an all-India basis will be evident from the fact that in contrast to Bombay (Khanolkar *loc cit.*) Poona has only 1 per cent rhesus negative population (Bird, 1946).

Maternity and child welfare — The problem of maternity and child welfare has been a subject of considerable investigation, although nothing constructive has been evolved and the difficulties do not appear to be any nearer solution today than it was at the beginning of the period of the present review. However, maternal mortality appears to have declined to some extent during this period in almost all the states of India. Foetal and neonatal death rates also show a slow improvement. In an informal symposium in December, 1949, held in New Delhi the problem has been widely discussed and the formation of a central maternal and child welfare committee suggested (Pandit, 1950). From all available reports it

appears that a lack of trained personnel is the great handicap in the achievement of satisfactory results. The infant mortality rate has been reduced from 233 in 1943 to 115.5 in 1948 in Delhi (Grewal, 1950), from 207.9 to 136.7 between 1944 and 1948 in West Bengal, from 110 5 to 91 9 between 1945 and 1947 in Assam (Devi, 1950), and from 178.5 to 128 1 between 1935 and 1948 in Madras (Rajamanikkam, 1950). However, Rao's (1950) figures from Mysore show little improvement in infant mortality during the 10 years preceding 1948 Improvement in maternal mortality is less striking, and has been about 1 per 1,000 births between 1935 and 1948 (Rajamanikkam, 1950) Mysore seems to have made considerable improvement during the 10 years period ending in 1948 and can boast of the lowest maternal mortality figure in India (3 7 in 1948 — Rao, 1950). This author, however, claims that the maternal mortality is 3½ times less among cases confined by trained midwives than among those attended to by *dhais.*

In an interesting communication Waters (1946) points out the influence of the economic and social status on still-births and neonatal deaths. He found that the overall still-birth rate of the hospitals of Bombay varied from 57 to 83, while the urban neonatal death rate was calculated at 80 per 1,000 live births. He also found that still-birth and neonatal death rates varied as follows . in emergency admissions 99 and 144, in booked hospital cases 19 and 30, and in nursing home cases 21 and 11 respectively.

Physiology of pregnancy — Little fundamental research has been done on this subject during the period under review Nayer (1940) studied the calcium and phosphorus level of the plasma in this condition Unfortunately the findings of these two authors show some amount of disagreement Nayer (1940) observed that serum phosphorus does not change throughout pregnancy except for a sudden slight rise during the last week of gestation. However, this interesting finding has not been studied in a proper manner and the conclusions must be accepted with caution until planned experiments in this direction are undertaken.

Bathiwalla (1948) worked on carbohydrate metabolism in normal pregnancy and found that the fasting blood sugar level falls and starving urinary sugar level rises as pregnancy advances There is a reduction in the tolerance for sugar during pregnancy. This confirms the findings of workers elsewhere This author however made one original observation which still awaits confirmation. He observes that pregnancy glycosuria is attended with a low level of ascorbic acid and calcium in the blood, and that administration of these substances to a pregnant glycosuric subject may reduce and even cure the glycosuria On the basis of data from a small series of animal experiments the author concludes that the beneficial effect of vitamin C and calcium is owing to an increase of renal threshold. It is a pity that this important observation has not been followed up. *Obstetric shock* formed the subject of study by Mitra (1940). Mitra pointed out that 2.4 per cent of

maternal deaths were due to shock unaccompanied by haemorrhage, retained placenta or rupture of the uterus.

Anaemias in pregnancy—Unlike toxaemia of pregnancy anaemias complicating pregnancy have been a subject of more systematic investigation. Normal haemoglobin values have been worked out by Rao (1938), Upadhyay (1944) (52 cases), Ghosh *et al* (1948) (359 cases), Kothari and Bhende (1949) (19 cases). Findings of these authors indicate that the average normal haemoglobin value in pregnant women is 10 g per cent.

The importance of anaemia in pregnancy as an obstetric problem has been reviewed by many workers. Mitra (1942) finds that this condition is responsible for 34 9 per cent of maternal deaths Ray (1942) observed that in a series of cases where the haemoglobin level was more than 40 per cent (Standard not mentioned) there was no maternal mortality, while in patients with less than 40 per cent haemoglobin the mortality rate was about 7 per cent. In an excellent memorandum of the India Research Fund Association, Napier and Neal Edwards (1942) summarise the results of investigations up to 1942 These authors call attention to three important types of anaemia seen commonly. These are (i) the microcytic hypochronic anaemia (ii) macrocytic hyperchromic anaemia of the nutritional macrocytic anaemia type, and (iii) macrocytic hyperchromic anaemia of haemolytic type While considerable advance has been made during the period under review in the elucidation of the nature of anaemias found in pregnant women, investigations in other aspects of the problem are singularly deficient. Metabolism studies have not been made. Chatterjee (1938) studied the cholesterol metabolism but his findings have not been confirmed by other workers (Nayer, 1940), Kothari and Bhende (1949). Chatterjee (1938), Nayer (1940) and Kothari and Bhende (1949) recorded a reduced concentration of serum proteins in this condition, but the nature of the change in protein fractions and the electrophoretic pattern of the plasma proteins in pregnancy anaemia still require to be studied.

In the therapy of anaemia of pregnancy little advance has been made since the discovery of folic acid The effect of crude and proteolysed liver extracts and folic acid has been studied by several workers of whom the work of Das Gupta (1946) is the most comprehensive.

Toxaemias of pregnancy—During the period under consideration sporadic researches have been undertaken in order to promote a better understanding of the subject. However, these have caused but little advancement in knowledge. Mitra (1948) studied the incidence of toxaemia in relation to the meteorological conditions prevailing in Calcutta in different seasons of the year.

Biochemical studies on the blood in 148 cases of eclampsia were undertaken by Mudaliar, Nair and Menon (1940). These authors came to the conclusion that eclampsia is attended with hypoglycaemia and hypercholesterolaemia.

Lipid metabolism has also been a subject of investigation. Mukherjee (1950) found that besides hypercholesterolaemia which was also observed by Mudaliar *et al.* (1940), a disturbance of phospholipid metabolism is also present in toxaemia of pregnancy. Plasma lipid phosphorus rises above the normal level in pregnancy, but rises even more in pregnancy toxaemia The lipid phosphorus level was correlated with oedema and it was observed that the mechanism of phosphorylation is disturbed in toxaemia of pregnancy.

Toxaemia of pregnancy is a wide subject Some advance has been made during the period under consideration in the understanding of the subject However, much remains to be done and a concerted effort in this direction is necessary.

New-born infant — Haematological studies on new-born infants have been done by several workers during the period Of these those of Mukherjee (1940), and Mukherjee (1944) are based on planned experiments The findings of these workers are very similar and appear to agree with those of the workers in western countries Mukherjee (1944) studied the haematological status of premature infants as well and found that these babies possess less haemoglobin and R B C at birth than full-term infants He however showed that by the end of the week these babies have less haemoglobin and red cells than normal babies.

Gynaecology — Numerous papers of clinical interest in gynaecology have appeared during this period Unfortunately however no original or outstanding observation has been made

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VI. METABOLIC DISEASES AND NUTRITION

It is proposed to give a bird's eye view of the progress made in the above speciality during the twelve year period (1938-1950) In reviewing the literature, some difficulties were felt, particularly in the selection of the material which strictly fall under the description — metabolic diseases and nutrition. Several important contributions on the biochemical aspects and the physiology of nutrition were severely left out with the idea that they will be dealt with elsewhere. The reviewer has tried to include the appropriate contributions by Indian workers

1. *Anaemias*: Numerous papers have been published on macrocytic and microcytic anaemias and the response to various treatments, like iron, liver preparation, folic acid and vitamin B_{12} with or without improving the "normal and habitual" diet.

Sankaran and Rajagopal (1938) have observed, that the daily administration of ferrous sulphate to girls and young women living in educational institutions in south India, produced a rise in the haemoglobin content of the blood In one institution from an initial value of 16 35 g per cent haemoglobin rose to 20.34 g and in another institution from 13 69 g to 18 44 g The rise was obtained *in 14 days and subsided* within two months of cessation of administration of iron. Napier and Majumder (1938) studied the haematological picture among pregnant tea garden coolie women in Assam The anaemia was of two types, microcytic hypochromic and macrocytic hypochromic The former type was a common among non-pregnant coolie women and was due to iron deficiency and low calcium and vitamin C in the diet The macrocytic anaemia was associated with deficiency of vitamin B complex It is known that there are cases of idiopathic iron deficiency hypochromic anaemia, where iron and liver are the proper remedies. Subba Row and associates (1938) have continued studies on the principle in liver effective in pernicious anaemia Mitra (1939) has found in pregnant women in two industrial centres in Assam, that the haemoglobin value in blood were low and there was a serious deficiency of available iron McRobert, Reddy and Subramaniam (1940) mentioned some histamine resistant cases of achlorhydria associated with anaemia. After appropriate treatment for anaemia gastric HCl reappear Srikantia, Rao and Rao (1940) have observed a marked increase in corpuscular glutathione in anaemias and particularly in anaemias due to ankylostomiasis Jacobson and Subba Roy (1941) have prepared a liver fraction by precipitation from an alcohol eluate of liver by reineckate and acid and subsequent treatment with charcoal and elution of the adsorbate with phenol. Further treatment of the phenol eluate with organic precipitant gave from 3 to 10 mg of amorphous organic products from 100 g of liver and a dose of 7 mg together with small quantities of the accessory product at an excellent therapeutic effect in cases of pernicious anaemia An investigation of 103 cases of macrocytic anaemia of pregnancy was carried out

in Madras by Mudaliar and Menon (1942). The anaemia was usually macrocytic and hyperchromic but in a few cases was macrocytic orthochromic or hypochromic. In the great majority, good results were obtained with parenteral liver therapy together with iron by mouth but blood transfusion was necessary in some cases. Where cases could be followed up, a normal blood picture was invariably found in 2 to 3 months after labour without further treatment.

Bagchi (1943) has analysed the data on 107 cases of anaemia in pregnancy in Calcutta The majority of them were macrocytic or normocytic, orthochromic or hyperchromic anaemia, only about 10 per cent of cases were of hypochromic anaemia Treatment was with iron injection of liver extract and blood transfusion. Taylor and Chuttani (1945) have brought evidence that anaemia is about 22 times more common in vegetarian than in non-vegetarians The anaemia, differed in the two groups, being macrocytic in the vegetarians, and hypochromic and normocytic or slightly macrocytic in the meat eating groups No case of nutritional macrocytic anaemia was seen in a meat-eater. Hynes, Ishaq and Morris (1945 *a*, *b*) working with Indian soldiers in Peshawar have found that anaemia was common and mostly of the hypochromic type and easily curable with iron A daily dose of 6 grains of ferrous sulphate produced marked improvement. The same workers (1945 *b*) suggest that though the iron content in the diet was about 60 mg most of it was present in a non-assimilable form and the anaemia responded to ferrous sulphate therapy Hynes, Ishaq, Morris and Verma (1946) working among Indian Army recruits have shown that anaemia was more common among 1/3 of the men whose muscle development was poor than in 2/3 whose muscle development was moderate Anaemia was least common in men with poor subcutaneous fat and most of the anaemia was normocytic and normochromic but there was usually evidence suggestive of iron deficiency Hynes, Ishaq and Verma (1946) have studied the effect of different diets and iron medication on the nutritional anaemia of Indian army recruits. Their conclusions are interesting. All anaemia which was considerable initially, improved under the influence of army diet, but without iron treatment, the severe cases were not cured within six months. Men on the lacto-vegetarian diet, without iron, responded less well than on other diet. The meat diet was slightly but not significantly superior to the standard diet. A daily dose of 3 grains of iron cured all anaemia in four months and 6 grains did not hasten recovery. Dasgupta, Ganguly and Chatterji (1946) have studied the effect of folic acid on nutritional macrocytic anaemia. Of the 8 cases studied, 7 showed marked improvement and 1 slight improvement. The improvement was preceded by reticulocytosis. The authors note that in properly selected cases of nutritional macrocytic anaemia, folic acid was not found to be inferior to potent liver extract. Patel (1946) has shown that in the treatment of nutritional macrocytic anaemia, refined liver extract given in relatively small doses was as good as crude liver extract given in larger doses. Patel and Bhende (1949) have described the changes in the pyloric region of the stomach in a case that died

of tropical macrocytic anaemia Karamchandani (1948) has given an account of the treatment of some cases of sprue with free folic acid or folic acid conjugates, with good reticulocyte response and general clinical improvement. Goodall, Goodall and Banerjee (1948) have recorded that folic acid in 10 mg doses twice daily produced prompt improvement in the blood picture and general condition in eight out of ten cases of nutritional anaemia It was more potent than liver in similar cases. The patients had normoblastic or megaloblastic bone-marrows and diamorphic peripheral blood Patel (1948) has treated two patients of tropical macrocytic anaemia with crystalline anti-pernicious anaemia factor (vitamin B_{12}) A single injection of 80 μg was given Both the cases showed increase in red cells and white cells count and in haemoglobin and there was reticulocyte response in five days and six in five days and six days respectively Patel and Bhende (1949) treated six cases of tropical macrocytic anaemia with synthetic folic acid. Basing their findings on blood count and bone-marrow picture, they report improvement in four cases and delayed response in one case Dasgupta, Chatterji and Mathen (1949) have reported that folic acid in the doses of 20 to 30 mg a day is a potent haemathinic in the treatment of macrocytic anaemia of pregnancy. Diamorphic anaemia does not react well to folic acid Kothari and Bhende (1949) have submitted a preliminary report on 40 cases of so-called pernicious anaemia of pregnancy They found that the only reliable criteria for the diagnosis of the condition is the presence of a true megaloblast in the peripheral blood and in the bone-marrow Ramalingaswami and Menon (1949) studied the role of folic acid in nutritional macrocytic anaemia Oral administration of the vitamin produced striking clinical and haematological improvement, similar to those obtained by crude liver extract on intra-muscular injection Gokhale and Chitre (1950*b*) studied the plasma protein levels of 40 healthy adult males and in 27 cases of macrocytic anaemia, 25 cases of microcytic anaemia and 7 cases of normocytic anaemia using Howe's method. They conclude that there were no significant variation among them. Plasma albumin levels showed a rise when the treatment was effective. Ramalingaswami and Venkatachalam (1950) carried out haematological studies on healthy adult males in south India at high altitude. There was no appreciable difference in the mean erythrocyte count and haemoglobin value from those recorded in the other parts of India at sea-level. Mean erythrocyte volume was increased There was distinct relative lymphocytosis with a corresponding decrease in the neutrophil polymorphs

2. *Peptic ulcer*: The aetiology of peptic ulcer remained obscure. Some attempts have been made by several Indian workers to find whether any relations exists between dietary deficiency and peptic ulcer M N. Rao, (1938*a*, *b*, *c*) has found an increase in bisulphate binding substances in ulcer cases and hence a possibility of relationship with vitamin B_1 deficiency. He did not find any changes in vitamin C value of blood. Rao (1939) and Dogra (1941) lean to the view that peptic ulcer is more common among people whose diet contains excess of carbo-

xerophthalmia was cured but lesions of the peripheral nervous system persisted. Dhurandhar and Boman-Behram (1940) are of the opinion that pigmentation of the conjunctiva is associated with vitamin A deficiency Rajagopal (1941) has shown that in clinical cases of night blindness the performance of the dark adaptation test is poor and that large doses of vitamin A orally brings a rapid response both clinically and as judged by the dark adaptation test Lawrie, Moore and Rajagopal (1941) have confirmed the excretion of vitamin A in urine in certain pathological cases. Khan (1945) has carried out dark adaptation tests among the army personnel. Dark adaptation tests have been conducted by Hassan and Khanna (1947). The aetiology of phrynoderma as due to vitamin A deficiency has been questioned by Gopalan (1947) who brings in evidence that fatty acid and other deficiencies might be responsible for the condition Ramalingaswami (1948) has described 'nutritional diarrhoea' due to vitamin A deficiency.

Vitamin B_1 Rice diets and Beriberi have been discussed by Akroyd and Krishnan (1941*a*). The same authors (1941*c*) have described infantile beri-beri and mortality due to the condition in Madras State The treatment and prevention of vitamin B_1 deficiency in infants, forms the subject of a paper by Krishnan, Ramachandran and Sadhu (1945), Raman (1941) has discussed the optic nerve lesions in vitamin B_1 deficiency. Anasas in babies cured by vitamin B_1 is the subject of a paper by C. K. P Rao (1941). There have been experiments on urinary excretion of thiamine and riboflavin by 15 normal adults under different dietary conditions, with a view to evaluating nutritional status with respect to thiamine and riboflavin, lack of uniformity of results, however, forbids generalisation.

Riboflavin : Akroyd and Verma (1942) have described superficial punctuate keratitis due to riboflavin deficiency. Verma (1942) have successfully treated the cases with oral and parenteral administration of the vitamin. Verma (1944) has recorded beneficial results with riboflavin in cases of angular conjunctivites. Vulval lesions due to ariboflavinosis has been described by Vakil (1945) Gopalan (1946) has drawn attention to less well described conditions of ariboflavinosis like preputial ulcers, angular blepharitis and lesions at other mucocutaneous junctions.

Nicotinic acid and pellagra . Akroyd, Krishnan and Passmore (1939) have described lesions of mucocutaneous junctions and point out that all of them are not early signs of pellagra and nicotinic acid cured the condition only in two thirds of these cases. Akroyd and Krishnan (1938) have described a stomatitis and its relation to P-P factor. Sen Gupta, Choudhury, Chaudhury and Napier (1939) have observed cases of pellagra in Calcutta which showed dermatitis, had achlorhydria and anaemia, diarrhoea and stomatitis All improved rapidly on hospital diet supplemented by marmite. Nicotinic acid was not available at the time. Cases of pellagra and their treatment have been described in the Punjab by Bajaj (1939), in Vizagapatam by Raman (1940), in U.P. by Ahmad (1942), in C.P. by Batra (1942) and in Calcutta by Napier and Choudhury (1943). An outbreak of

pellagra in a rural area in Bengal and in Calcutta has been described by Napier and Choudhury (1943). An outbreak of pellagra in a rural area in Bengal and their treatment is described by Chaudhury and Chakravarti (1947). Scrotal dermatitis, stomatitis and allied conditions and Orogenital syndrome as manifestations of vitamin deficiency have been recorded by Karunakaran and Nair (1940) and Mitra (1943). Ramalingaswami, Menon and Venkatachalam (1948) have reported the occurrence of pellagra in children aged six months to three years Gokhale and Chitre (1950 *a*) observed the plasma protein composition in B complex deficiency states A decrease in albumin and an increase in globulin level was observed Wagle (1950) reported an unusual case of B complex deficiency The symptoms observed were pains in gravid uterus simulating threatened abortion, which had defied intensive treatment with uterine sedatives for long-period. The symptoms disappeared completely after three injections of B complex

Vitamin C. An outbreak of scurvy in a famine area in Hissar district and its management in the Punjab has been described by Khan (1942) A case of scurvy treated with tomato juice is recorded Lewis and Dutta (1942). A case of renal glycosuria associated with hypovitaminosis is described by Banerjee (1947). No evidence of renal disorder was found and the Glycosuria was thought to be due to vitamin C deficiency

Vitamin D Patwardhan, Chitre and Sukhtankar (1944 and 1945) have reported the fluctuations in the ionic products of calcium phosphates in blood serum in rachitic infants Dikshit and Patwardhan (1944) have made studies of the changes in the ionic products of calcium phosphates and alkaline phosphatase at the outset, progress and healing of rickets

Vitamin K: A method is described by Rahman and Giri (1945) in which a preparation of Russell's Viper venom in $CaCl_2$ solution is used as a source of thromboplastin This reagent was found stable and prothrombin time of several cases were estimated with this preparation Braganca and Rao (1947) have recorded that hypoprothromabinaemia produced by sulphathiazole in rats on a diet free from vitamin K can be cured by synthetic vitamin K.

7. *Miscellaneous*: Akroyd, Krishnan, Passmore and Sundararajan (1940) have brought out a valuable monograph on "The Rice problem in India". Some of the interesting points mentioned are, that the loss of nutritive value of milled rice is mitigated when it is parboiled, because such rice retains most of vitamin B_1 and nicotinic acid originally present in unmilled rice. Washing and cooking reduce these by fifty per cent Beriberi is endemic only in areas in which rice is consumed and tends to occur in those who consume diets in which the ratio of vitamin B_1 to calorific value is less than 0 25. Cochrane, Paulraj and Salmond (1940) have observed the beneficial effects of whole wheat diet in leprosy patients complaining of chronic nerve and bone pains Kirwan, Sen and Biswas (1941) and Kirwan,

and Bose (1943) have found that parenteral administration of vitamin A was very effective in curing keratomalacia cases. They are not convinced that conjunctival pigmentation and Bitot's spots are necessarily due to vitamin A deficiency. Biophotometer tests did not reveal vitamin A deficiency in most of them Bopaiya and Rao (1942) have stressed factors other than malnutrition as the primary cause of "Tropical ulcer". Rao, Colah and Kalle (1945) have extended the studies on tropical ulcer in Bombay Province and have said that good food, particularly rich in vitamin A and Ca can be recommended for mobilising the defensive forces of the body to promote rapid healing of the ulcer Several studies have been carried out in Calcutta on the 1943 "Bengal Famine". Aich, Chakraborty and Chandra (1944) have found that out of 7,000 starving sick destitutes, 50 per cent suffered from gastro-intestinal complications, 75 per cent from ulcers usually of the lower extremities and 55 per cent from malarial infections Treatment of cases of collapse by intravenous injection of glucose and peptone gave good results Chakravarty (1944) has studied the biochemical changes in blood in starvation cases Chari (1944) has commented on the frequency of oedema and the absence of specific vitamin deficiency signs in starvation cases Menon (1945) and Mohanty (1945) have also made clinical studies of starvation cases. Krishnan, Narayanan and Sankaran (1944) have discussed the preparation of a protein hydrolysate for the treatment of marution cases. They have now a papain digest of meat, containing 5 per cent protein hydrolysate and 5 per cent glucose and 0 85 per cent NaCl This was sterilised, tested for freedom from toxicity and allergic effects About 3,000 injections on 1,000 patients gave untoward effects only in a very few cases. Bhattacharya and Sen (1945) have made postmortem studies on cases that died of famine in Bengal. They observed a shrinkage and loss in weight in many internal organs and commented on the freedom from specific vitamin deficiency signs Karamchandani and Hyder (1946) have commented on the occurrence of sprue in Indian Army troops. Gopalan (1946) has described "Burning feet syndrome" due to pantothenic acid deficiency Menon, Tulpule and Patwardhan (1950) observed that marmite alone was ineffective in curing human phrynoderma which gingelly oil cured. Gingelly oil also rectified the lowering of iodine number of plasma fatty acids in this condition These workers postulate that this condition is a manifestation of essential fatty acid deficiency

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VII. TUBERCULOSIS

The subject may be conveniently divided under several heads as indicated below:

EPIDEMIOLOGY

The incidence, types of tuberculosis and factors in its causation continued to be studied by means of tuberculin tests and chest skiagraphy in selected groups of population in different parts of India (Benjamin, 1938, Riste, 1938, Cummins, 1939, Sanjivi, 1939; Cruickshank, 1939, Government of Bengal, 1939, Ray, 1941; Strain, 1941, Wig and Riyaz, 1941, Shah, 1942, Sahni, 1942, Lal *et al.*, 1943; Taylor, 1944; Ukil, 1944, Ghosh, 1944, Nayyar, 1944, Aspin, 1945, Misra, 1946; Ukil, 1948, 1949, Frimodt-Moller, 1949) The later surveys were gradually dealing with both infection and morbidity rates based on scientific planning, but no surveys could yet be undertaken on an all-India basis until later.

The technique of scientifically designed surveys began to be considered and several reports on the technique were published (Report of I R.F A Committee — 1940; Taylor, 1939, Lal, 1940 *a*, *b*, Benjamin, 1940, Chandra Sekhar and Sen, 1945, Ukil, 1948, and Ahmed, 1948)

Several papers on the determination of types of tubercle bacilli isolated from pulmonary and non-pulmonary lesions in man were published (Pandalai, 1940, Mallick *et al.*, 1942, Ukil, 1948). It is interesting to record that out of 221 strains tubercle bacilli isolated by Ukil and 141 strains isolated by other workers from non-pulmonary sources, all proved to have belonged to the human type. The incidence and types of osteo-articular tuberculosis were dealt with by Strain, (1941), Kini (1948), and Narasimhan (1948).

The question of silicosis and dust hazards was studied by Sen (1939 *a*, *b*), Ukil (1948), and Sikand (1949). In a survey on the relation of jute industry in Bengal, Ukil and Sen (1948) tried to find out the infection and morbidity rate in 4,816 mill operative (A), 1,107 relatives of workers living in the mill area (B) 1,100 relatives of workers living further out of the mill area (C) and 1,200 rural population, as contrast, living in distant villages (D). The highest infection and morbidity rates were found in the first group (A), progressively diminishing in groups B, C, and D. Both the infection and morbidity rates were highest in the spinning section of the mill, where the dust hazard was the worst.

The influence of sunlight on experimental tuberculosis was studied by Ukil (1938). The relation of climate to tuberculosis was dealt with by Bose (1945). The question of the application of actinotherapy and the role of solaria was dealt with by Jayaram and Sheriff (1938); Ahmed (1945) and Talwalkar (1948).

Clinical Pathology and Diagnosis

As is natural, a fairly large number of papers were published on the early recognition, standards and technique of diagnosis (including differential diagnosis) of pulmonary and non-pulmonary tuberculosis. Several papers on pathological concepts, instrumental technique and laboratory studies in the diagnosis, prognosis and treatment of tuberculosis were contributed. Papers on biochemical changes in blood were contributed by Reddy and Venkataramiah (1942), Billimoria and Jacoby (1945), Ray (1948) and Ray *et al.* (1949)

Attempts to standardise methods of diagnosis, prognosis and treatment were made by Ukil (1939, 1940-41, 1945), Sen (1939 *a*, *b*, 1948) and the Tuberculosis Association of India (Report of Classification, 1940) The after-results of cases discharged from institutions were studied by Barton (1939 *a*, *b*, *c*), Benjamin (1939, 1942, 1948) and Hamid (1946)

Association of Tuberculosis with Other Conditions

The adverse association of tuberculosis and Kala-azar was noted by Ukil (1939). The association of diabetes with tuberculosis was studied by Benjamin and Verghese (1940), Billimoria (1940) and Neogy and Ray (1944) The question of pregnancy and tuberculosis was studied by Ghosh (1940), Chowdhury (1944) and Ghosh (1948). Abdominal tuberculosis was studied by Viswanathan (1940), Anderson (1940), Ukil (1942), Frimodt-moller (1943), Misra (1946), Datta Gupta (1948) and Tribedi and Gupta (1941) Lymph node tuberculosis was dealt with by Cruickshank (1939); Osteo-articular tuberculosis by Strain (1941); Kini (1948); Narasimhan (1948), and renal tuberculosis by Basu (1949).

Several papers on non-tuberculosis pulmonary diseases like pulmonary abscess, bronchiectasis, bronchial asthma, "eosinophilic lung", hydatid disease, pulmonary amoebiasis, carcinoma and syphilis, endothelioma of the pleura and pulmonary moniliasis were contributed.

Treatment

The subject can be conveniently considered under the following headings :

Chemotherapy : Several papers were published on the use of gold therapy (Chatterjee and Ukil, 1938-39 *a*, *b*; Shrikhande, 1939 *a* or *b*; Sanyal, 1939), cadmium salts (Nizamuddin, 1942) and Promin (Benjamin and Frimodt-moller, 1946), but these soon yielded place to para-amino salicylic acid (Dempsey, 1948, Frimodt-moller, 1949 *a*, *b*; Ukil, 1949, Sahgal, 1949); and streptomycin, either singly or combined with PAS (Ray, *et al.*, 1948, 1949, Ray, 1948; Sanjivi 1949; Patel 1949 *a*, *b*, Sen 1949, Rao, 1949; Sen, 1949; Singh, 1949).

SURGICAL THERAPY OF PULMONARY TUBERCULOSIS

Several papers appeared on suction drainage, artificial pneumothorax — intra — and extra — pleural, including pneumolysis and complications of A.P.; phrenic exairesis, pneumoperitoneum, thoracoplasty, and pulmonary resection, under Indian conditions

TREATMENT OF OSTEO-ARTICULAR TUBERCULOSIS

Treatment of osteo-articular tuberculosis was dealt with by Strain (1941), Kini (1949), Narasimhan (1948), and Guha (1948)

PREVENTION

The public health aspects were emphasised in a number of papers (Frimodt-moller, 1938, 1939, Ukil, 1938, 1939, Ram, 1940; Patel, 1944, Lal, 1948 *a* or *b* or *c*; Viswanathan, 1948 *a*, *b*, *c*, 1949) The problem of wartime tuberculosis was dealt with in two papers Shah (1945), Joseph (1943 *a* or *b*, 1945). Tuberculosis in relation to Life Insurance was dealt with by Rao (1949). The social aspects of tuberculosis were considered by Banerjee (1948), Majumdar, (1948), and Buck (1948) The subject of after-care was dealt with by Mani (1939), Barton (1939 *a*, *b*, *c*), Hamid (1946), and Benjamin 1948 *a* or *b* or *c* or *d*) The subject of Rehabilitation was discussed by McDougall (1949), Chowdhury (1949), and Dwarkadas (1949).

B.C.G VACCINATION

Apart from other methods of prevention, a plea for the use of B.C.G. vaccine in immunisation was first made by Ukil (1946) in a paper giving an account of its use as far back as 1934. A co-ordinated programme on a nation-wide scale was evolved and introduced in 1948 Since then a number of papers on its preparation and trial appeared (Benjamin, 1948, Lal, 1948, Svendsen, 1948; Ranganathan, 1948, Gellner, 1948, Patel, 1949, Wig, 1949; and Frimodt-moller, 1949).

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VIII. CLINICAL ASPECT OF COMMUNICABLE DISEASES

MALARIA

The new synthetic antimalarial drugs have been extensively tried in India for their therapeutic and prophylactic values Paludrine investigations were carried out by Chaudhuri and Rai Chaudhuri (1946 *a*, *b*), Afridi (1947) and others. *Chaudhuri* (*1948*) drew attention to the existence in India of strains of *P falciparum* resistant to paludrine which have since been confirmed (Chaudhuri and Rai Chaudhuri, 1949) The drug was also found relatively slow in action Paludrine has been administered intravenously (Chaudhuri and Chakravarti, 1949, Mullick and Gupta, 1947, 1948) and intramuscularly (Chaudhuri *et al*, 1950) but these offer no advantage over oral therapy and parenteral quinine is the therapy of choice for the emergency treatment of malaria (Chaudhuri and Chakravarti, 1950). M 5943 or N_1 3.4-dichlorophenyl N_5 isopropyl diguanide, another diguanide derivative prepared by the Imperial Chemical Industries was relatively more toxic and the results were rather disappointing (Chaudhuri *et al.*, 1950*a*, *b*). The 4-amino-quinoline derivatives, chloroquine and camoquin have been found to terminate a clinical attack of malaria much earlier than other drugs (Simeons and Chhatre, 1947; Chaudhuri and Chakravarti, 1948, Chaudhuri *et al*, 1948) The slower response obtained by Patel and Mehta (1948) with camoquin was apparently due to the small dosage used Chaudhuri *et al* (1950) showed that clinical attacks of malaria could be controlled by single doses (0 5 g base) of chloroquine, camoquin and paludrine, the drugs being effective in that order A drug prepared from dehydrocholic acid and quinine sulphate was used by Chakravarty and Basu (1950) in the treatment of malaria. The advantages claimed are quicker absorption, better therapeutic results and less consumption of quinine alkaloid

For suppressive treatment of malaria weekly doses of chloroquine or camoquin gave better results than either paludrine or quinine (Chaudhuri and Poti, 1950, Chaudhuri *et al*, 1950, Patel and Mehta, 1948) These clinical results correspond to the experimental observation of Singh *et al* (*1949, 1950*) who compared the suppressive action of various antimalarial drugs against *P knowlesi* in *S rhesus* monkey and obtained markedly superior results with chloroquine and camoquin, the former being apparently the more effective of the two Favourable results in the chemoprophylaxis with mepacrine, paludrine or chloroquine were also reported by Adhikary (1947), Mullick (1948), Ray (1948) and Banerjea (1949). The last author found weekly doses of 0 1 g paludrine of little value but bi-weekly doses were effective. Ray (1948) observed an overall improvement in the death rate, infant mortality and birth rate in addition to the control of malaria These works show how malaria can be controlled by the regular administration of drugs in the transmission season when mosquito control is not possible

A detailed account of the recent antimalarials has been given by Chaudhuri and Dutta (1950) and Jaswant Singh (1950).

Insecticide spray (DDT and BHC) has been undertaken in various parts of India with generally satisfactory results (Afridi and Singh, 1947; Nair, 1948; Singh and Kariapa, 1949, Singh and Singh, 1949, Viswanatham and Rao, 1947, 1948, 1949; Viswanatham, 1949; Viswanatham *et al*, 1949, Ramkrishnan *et al.*, 1948, Adhikari and Ganguli, 1949, Hazra, 1948, Vedamanikkam, 1949, Srivastava, 1950).

The Malaria Institute of India carried out field trials with insecticides and found that DDT applied at 50 mg per sq. ft. (1.76 oz. per 1,000 sq ft) and gammexane suspension at 1 mg gamma isomer per sq ft. (0 035 oz. per 1,000 sq. ft.) gave mosquito control for 10 or more days Gammexane appeared to be more effective (I.R F.A. report, 1948). Suspension of DDT was found to be more satisfactory than solution for application to walls of houses by Sunderaraman and Peffly (1949)

Singh and Nair (1950) described abnormal forms of *P vivax* in a 7 month old child in Delhi. Viswanatham (1944) found antimortem clot in the sagittal sinus in several cases of cerebral malaria Nandi (1949) reported 4 cases of *P falciparum* and one of *P. malariae* infection with unusual manifestation. The last one presented as a case of "acute abdomen" and all responded to quinine therepy P *knowlesi* infection in monkey has been successfully treated with thiazole derivative of sulphanilamide or stilbamidine (Patel, 1944, Das Gupta and Siddons, 1944) Singh *et al.* (1949, 1950) found trypanosomes in addition to plasmodial sporozoites in the salivary glands of *C fatigans* fed on sparrows and successfully transmitted *P knowlesi* to two monkeys by injection of infected glands of *A. stephensi* and *A annularis*

KALA-AZAR

During the period under review kala-azar was for the first time successfully transmitted to human volunteers by the bite of infected sandflies (Swaminath, Shortt and Anderson, 1942). This corroborates the epidemiological and other evidence that kala-azar is naturally transmitted by *Phlebotomus argentipes* in India and presumably by other species of sandfly elsewhere. The introduction of a complement-fixation test for the diagnosis of kala-azar is another important contribution from India (Greval, Sen Gupta and Napier, 1939, Sen Gupta, 1943*a, b*)

The antigen is prepared from acid-fast bacilli but the reactions are highly specific, a positive reaction being obtained in 93 per cent of cases as early as 3rd week of the disease. Newer modifications of *formol-gel* and *antimony test* have been described, one of which can be performed with reconstituted sera from blood soaked in blotting paper (Raghavan and Prakash, 1949; Raghavan, 1949). Chakravarty, *et al.* (1949*a, b*) carried out a series of biochemical investigation in kala-azar

and demonstrated a depression of adreno-cortical and hepatic functions which could be correlated to some of the disease. The changes of plasma proteins were studied in recent years by Chakravarty *et al.* (1949 *a* or *b*) and Chakravarti (1950). In the field of treatment the newer aromatic diamidines were tried extensively and stilbamidine was found to be the most portent drug, being effective even in resistant cases of kala-azar (Napier, Sen Gupta and Sen, 1942). The drug is however toxic and almost invariably leads to a late neurological sequelae (Napier and Sen Gupta, 1942, Sen Gupta, 1943 *b*) Pentamidine isethionate is free from this effect and is less effective (Sen Gupta, 1948). Hydroxystilbamidine is however almost as effective as stilbamidine but free from its neurological sequelae (Sen Gupta, 1949, 1950). Sodium antimony gluconate which is identical with the German preparation solustibosan (first tried in India by Napier, Chaudhuri and Rai Chaudhuri in 1938) when used in a higher dose was found to be immediately curative in over 90 per cent but the relapse rate was rather high (Sen Gupta and Chakravarti, 1945 *a*, *b*). N-methyl glucamine antimoniate, a pentavalent antimonial of low toxicity, was used with good results, the excretion of antimony after the administration of the drug was rather rapid (Sen Gupta, 1950, Chakravarti and Sen Gupta, 1950) Das and Sen Gupta (1950) reported a fatal relapse of kala-azar after splenectomy and discussed the role of splenectomy in the treatment of kala-azar. In kala-azar complicated by cancrum oris or agranulocytosis (Das Gupta and Sen Gupta, 1943, Sen Gupta and Chakravarty, 1945 *a* or *b*, Banerjea, 1949) penicillin in addition to the usual treatment saved many patients (Sen Gupta and Chakravarty, 1946, 1947) For prophylaxis, 3 oz DDT per 1,000 sq. ft gives control of *P argentipes* for 7-8 months (I R F A report, 1948)

CHOLERA

Statistical evidence has been produced to show that cholera incidence was very significantly less in persons protected with a single dose of *Inaba Ogawa* vaccine as compared to the unprotected group (I R F A Report 1944) Ahuja and Singh (1948) found a rise of bactericidal titre after inoculation with *V. cholerae* This was parallel to the passive protection given by serum to infected guinea-pigs but the agglutination titre was not a good index of the bactericidal titre It appears that there is a good cross-immunity to *Inaba or Ogawa* infection when either strain is used alone for inoculation Storage of drinking water in copper vessel for at least 4-6 hours has been shown to be lethal to cholera vibrio (Bose and Chakravorty, 1948). Srivastav[illegible] extracted from *V* [illegible] with the ordinary cholera vaccine. Singh *et al.* (1950) however observed that the polysaccharide fraction though exerting considerable protection to mice was rather less effective than the usual vaccine. Veeraraghavan (1949) has devised a simple liquid medium which produces heavy growth of *V. cholerae*. Others used hydro-

lysate casein medium for culture and preparation of vaccine of cholera and plague (Sokhey *et al*, 1950, Seal, 1950, Seal and Mukherji, 1950). The antigenic relationship of different types of *V cholerae* was studied by Singh and Ahuja (1950). Mehta (1950) presented a statistical analysis of cholera mortality and seasonal incidence in Hyderabad State for 45 years (1904-1948).

Several workers have reported better results in cholera patients treated with sulphaguanidine in addition to usual treatment as compared with the control series (Seal, 1947; Lahiri, 1945, 1948, Pasricha *et al.*, 1947 *a*, *b*, Misra, 1944) Bhatnagar *et al.* (1948 *a*, *b*) had very favourable response with formo-sulphathiazole. Chaudhuri *et al* (1950) found that chloromycetin caused rapid clearance of vibrios from the stools of cholera patients but had no effect on the mortality rate.

Plague

The most important work in plague is the therapeutic success with streptomycin and sulpha drugs Sulphonamides considerably reduced the death rate and of these sulphadiazine is the drug of choice (Sokhey and Wagle, 1946, Simenos and Chhatre, 1946). The value of streptomycin was clearly demonstrated by Sokhey (1947), Karamchandi and Rao (1948) and in the Calcutta outbreak of plague (Dutta Gupta, 1948), in United Province 155 cases were treated with streptomycin and sulphadiazine with 6 deaths; five of these were admitted in a moribund state (Ghosh, 1950). Streptomycin was also used (0 66 mg 4 hourly till improvement, total — 15.3 — 38 mg) in 6 cases of pneumonic plague, 3 primary and 3 secondary, with recovery in five. All the recovered cases received anti-plague serum and some had sulphamerazine in addition. (Wagle and Bedarkar, 1948).

Sokhey and Habbu (1950) found oral aureomycin and chloromycetin effective in experimental plague in mice. The results were similar to subcutaneous streptomycin but the total dosage used was minimum for streptomycin and maximum for chloromycetin Sokhey and Wagle (1946) showed that the degree of septicaemia in plague was the most important single factor in prognosis. Seal (1949) described local outbreaks of pneumonic plague in Calcutta and Gaya which terminated spontaneously. Simeons and Chhatre (1946) did not notice much difference in mortality in the inoculated and unprotected groups and stressed the anti-flea (specially DDT) and rat-baiting measures in the control of an epidemic. Patel and Rebello (1948) however, observed in a field of investigation that inoculation reduced the mortality rate by about 20 per cent, the reduction in attack rate being less marked.

Typhus and Typhoid Fever

The incidence of typhus fever is being more widely recognised in India. All the types have been reported — Scrub typhus being the commonest (Chaudhuri and Chakravarti, 1949, Krishnan *et al.*, 1949). The greatest prevalence was in rainy

season when the vector of Scrub typhus *T deliensis* was found in abundance; 20 per cent of the *R rattus* caught were infected with *R. orientalis* (Krishnan *et al.*, *loc. cit.*). Para-aminobenzoic acid was used in typhus fever with some success but it has been replaced now by the more potent drugs chloromycetin and aureomycin (Krishnan *et al*, 1950). Khan (1950) described 400 cases of Scrub typhus with 11 deaths. Several cases were successfully treated with aureomycin. Chaudhuri (1948) from a study of the protein and carbohydrate metabolism in typhoid fever has stressed the importance of a high calorie high protein diet for its management Chhetri and De (1947) described a complication of jaundice with gastric haemorrhage and melaena (due to hypoprothrombinaemia) in cases of typhoid fever kept on a poor nourishment Napier *et al* (1942) reported the Widal reaction in typhoid cases and control subjects in Calcutta. The titre of H-agglutinin in the latter rose to 1 : 100 in 15 per cent and 1 200 in 7 per cent while that of O-agglutinin rose to 1 · 200 in 23 per cent and to higher dilutions in 14 per cent. In view of these findings they think that in a suspected case H-agglutination in 1 : 200 and O-agglutination in 1 · 400 only should be considered as very suggestive of enteric fever.

Helminthic Disease

Mukherji and Bhaduri (1945, 1947) found the indigenous drugs Butea and Embelia and Kamala effective for round worm but not for hookworm and tapeworm. They also described a case of gnathostome infection of the eye with orbital cellulitis which cleared up when the worm was removed Seven cases of cysticercosis, five confirmed by histology were reported by Raman *et al.* (1950 *a*). Guha (1949) described a fatal case of massive ascaris infection with numerous worms emerging from nose, mouth and tooth and a rent in the stomach There were also pockets of worms in the liver spleen and pancreas Guinea-worm infection has been reported in dogs and it is pointed out that this may pollute the water of ponds (Sharma and Hussain, 1946). Raghavan and Krishnan (1949) reported *W malayi* infection in an island of Madras Presidency, the probable vector being *Mansonioides annulifera.* The incidence of hookworm infection in the Jharia coal-field settlement area was surveyed by Mukherji and Mathen (1950). The ascaricidal content of oil of chenopodium has been shown to undergo progressive deterioration (most rapid in the first year) on storage at 0·98°F (Mukherji and Ghosh, 1943). Hetrazan caused disappearance of microfilarae from peripheral blood but had little effect on the clinical manifestations of the disease (Raman, 1950 *b*, Bhaduri, 1950).

Leprosy

Dharmendra and Shantra (1945) reviewed the results of leprosy survey and reported that the incidence varied from 0 17 to 6 61 per cent of the population in different parts, a higher total incidence being associated with high incidence in children and of lepromatous cases. A high incidence (1.3 per cent of a population

of 2,26,122) was also reported in Orissa specially in the humid coastal districts (Verghese and Rath, 1942). There are probably 20,000 cases of leprosy in the city and suburbs of Calcutta the incidence rate being 0.3 — 1.65 per cent (Sen, 1949). In Bombay more than half of contacts of leprosy showed clinical evidence of disease and a quarter was bacteriologically prositive. (Figueredo and Desai, 1949). Cochrane and Rajagopalan (1943) showed that contact was the determining factor of the incidence of leprosy in a family and hereditary susceptibility was not of much importance. Cochrane *et al.* (1945) produced positive lepromin reaction in monkeys inoculated with human leprosy material after preliminary splenectomy but Dharmendra and Mukherji (1944) failed to confirm this. Dharmendra (Napier, 1943, Dharmendra and Jaikaria, 1943) has shown that the protein constituent of the bacilli in leprous nodules is responsible for the lepromin reaction and by using this fraction he could enhance the early response while almost abolishing the late nodular reaction The reactions in non-contacts was also significantly reduced. He also reported good result with nucleo-protein extract in phosphate buffer There was a tendency to increased reaction to lepromin when lepromatous lesions subsided but a fully positive reaction was rare. Relapse was less frequent in cases with increased response (Dharmendra and Mukherji, 1947). The lepra bacilli were seen to lose their acid fastness on exposure to sunlight. This is another point of difference from rat leprosy organisms which do not possess this property (Dharmendra and Mukherji, 1949). The false positive serological reaction for syphilis that may be found in leprosy is discussed by Singh (1949).

Successful treatment with sulphone group of drugs has been reported in India by Cochrane *et al.* (1949), Dharmendra (1948, 1949, 1950 *a*, *b*) and others. The parent compound diaminodiphenyl sulphone (DDS) was previously given up as too toxic, but Cochrane (1949) has lately shown that it produces good results when used in small doses. It may be given up by the oral or intramuscular route and is usually fairly well-tolerated in the small doses now used. It has an extra advantage of being cheap. Sulphetrone and diasone injections gave good results and were more economical (Cochrane, 1948, Chatterjee, 1949; Dharmendra *et al.*, 1950). Neural cases also improved with sulphetrone injections (Dharmendra and Chatterjee, 1950)

Diphtheria

De and his colleagues (1947) reported success with local and parenteral penicillin therapy in diphtheria. Ghosal (1944) examined the types of organism in diphtheria and found that mitis was the predominating type in Calcutta. It produced more severe disease here than in England.

Weil's Disease

Authentic cases of Weil's disease have been reported in India during the last few

years. Das Gupta (1938) confirmed the diagnosis in 40 to 50 cases and found no association with any particular occupation He found the infection rate in rat population to be low (less than 10 per cent) in Calcutta, though higher in the dock area. Lahiri (1943) in Bombay found 12 per cent of the rats infected.

Amoebiasis

Cases of pulmonary amoebiasis and amoebic granuloma or amoeboma of the intestine, one of which was associated with carcinoma have been reported by Chaudhuri and Chakravarti (1946), Chaudhuri and Rai Chaudhuri (1946 *a*, *b*). Reddy and Thangavelu (1948) also described cases of amoeboma of the intestine and other complications of amoebiasis including amoebic brain abscess, cutaneous amoebiasis, monolobular cirrhosis following biliary obstruction due to an abscess of liver and suppurative pericarditis

Koshy (1950) has also recently reported a case of amoebic abscess of the brain with amoebic ulcers of the intestine Ghosh and Mukherjee (1950) described cutaneous amoebiasis of the peri-anal region and around colostomy wound. Plasma protein changes in amoebiasis was studied by Chakravarti

Rabies

Ahuja (1950) reviewed the work in India since 1900 The Paris strain of fixed virus gave better results than the Kasauli strain and has generally substituted the latter in India He attributes paralytic accidents to the living virus used before and the quantity of the nervous tissue injected Two cases of rabies in the tiger have been described from Pasteur Institute, Shillong (Pandit, 1950). Both the animals had mauled several persons and it is suggested that rabies may be responsible for the "man-eating propensity" of the tigers

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